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Figure 1 - Healthy Nucleus Pulposus Tissue

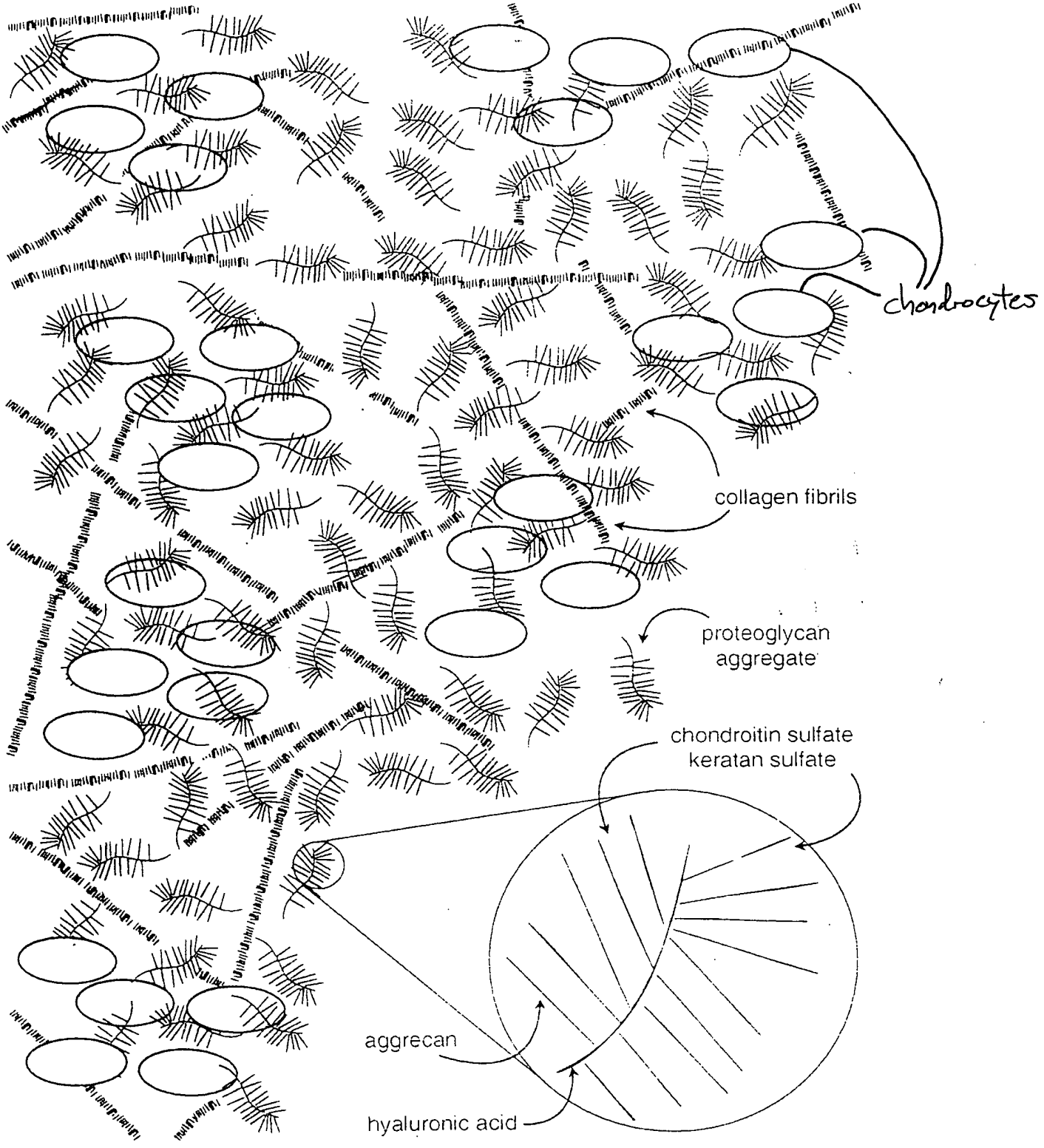


Figure 2: Cross-linked Matrix

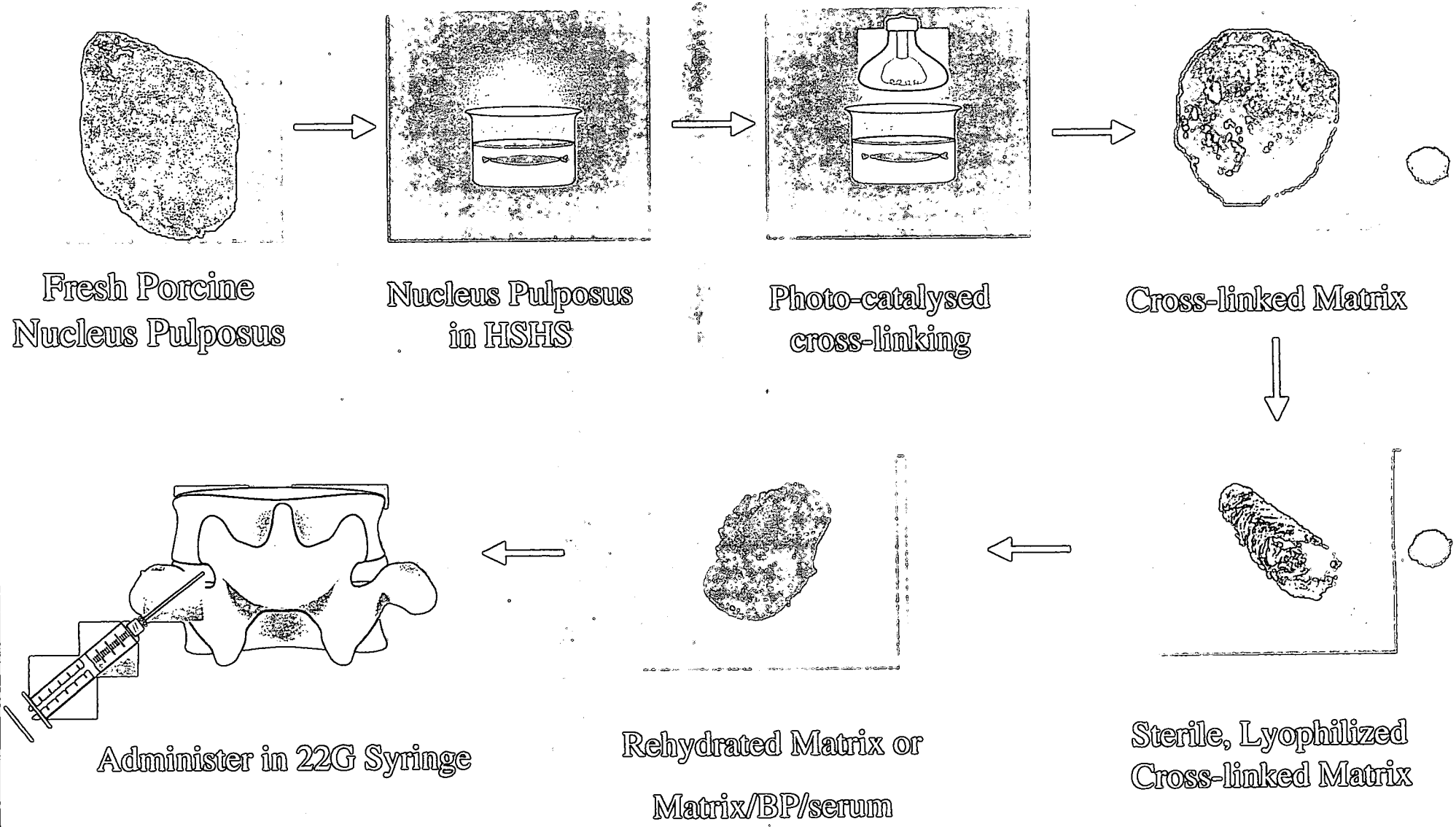
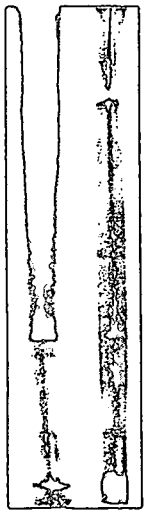


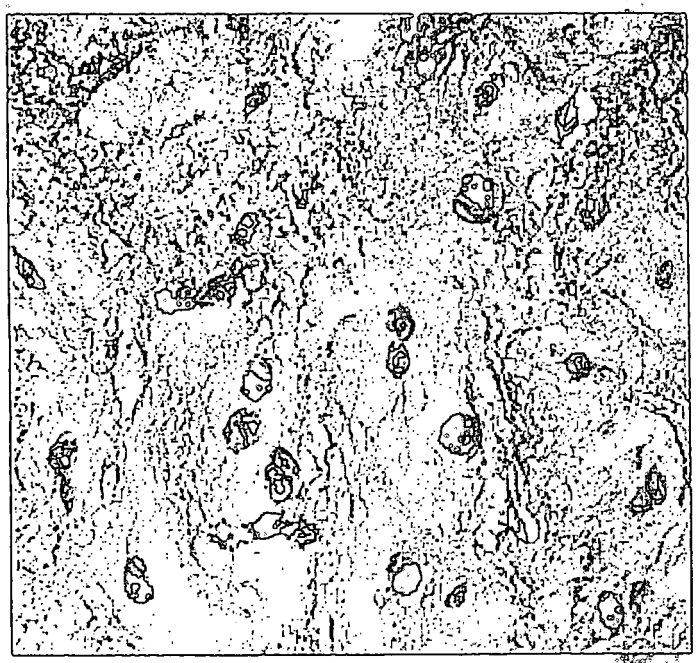
Figure 3



A B

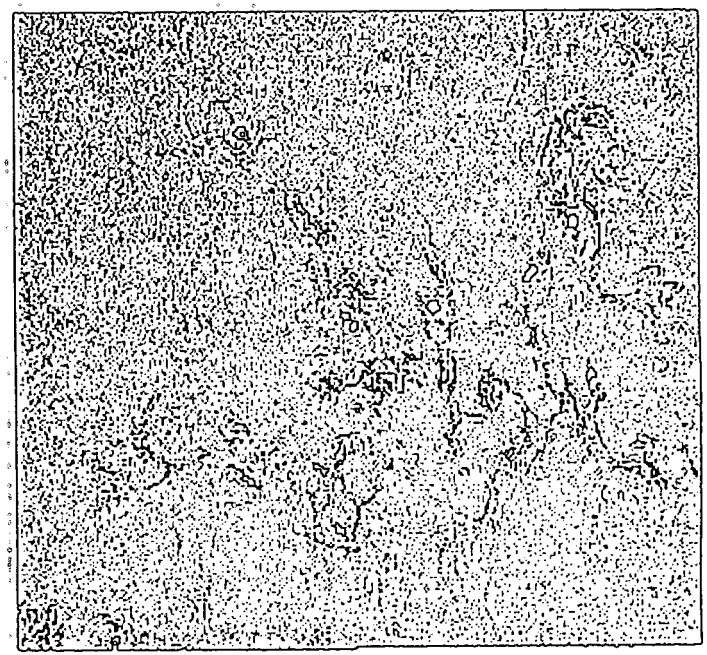
- Lane A: Non cross-linked control shows substantial protein extraction
- Lane B: Cross-linked matrix demonstrates reduced protein extraction

Figure 4: Comparison of Fresh Porcine Nucleus Pulposus versus Cross-linked Matrix



Fresh Nucleus Pulposus

- round, nucleated chondrocytes
- intact pericellular matrix "nests"



Cross-linked Matrix

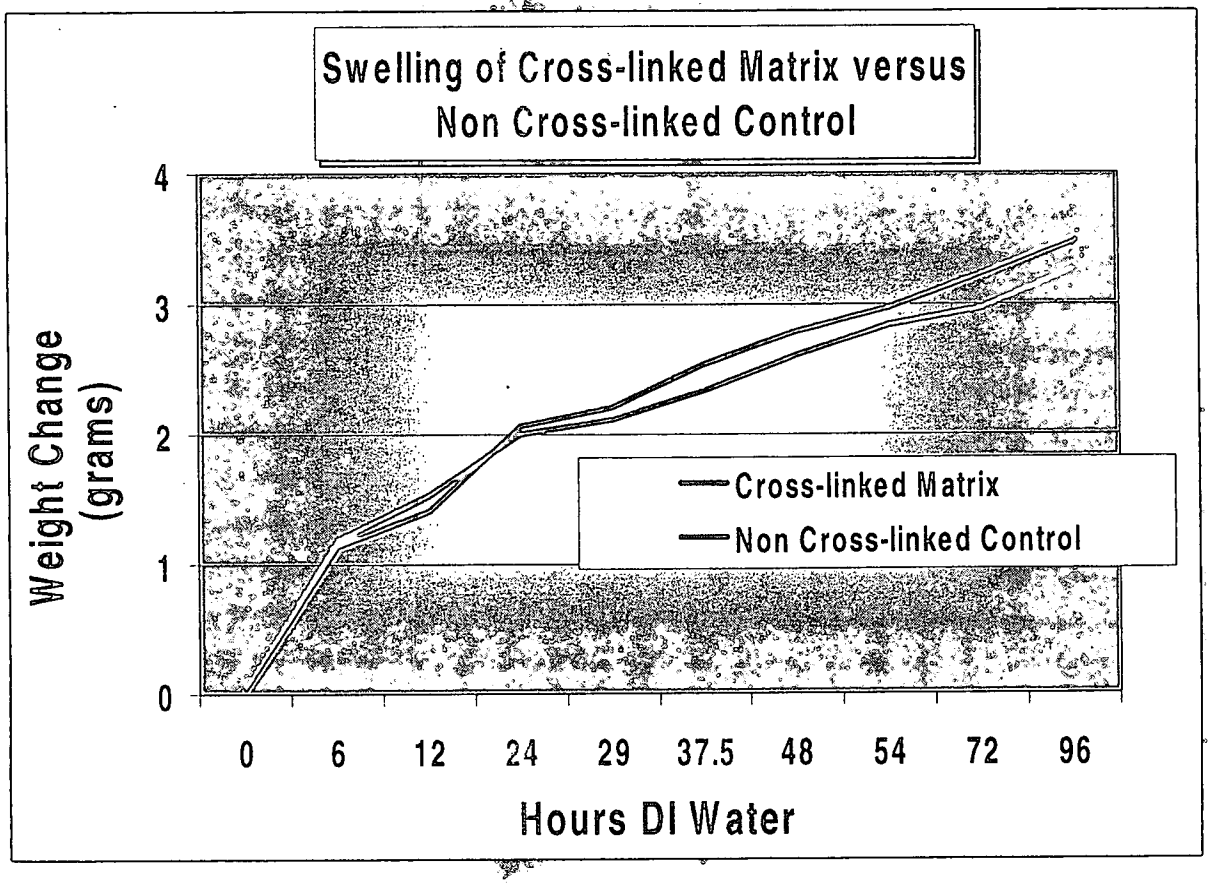
- disrupted, crenated cell fragments
- minimal cell membrane material
- further isopropanol sterilization

Figure 5

A B

- Lane A: Pepsin digests of non cross-linked control react with Type II collagen antibodies
- Lane B: Pepsin digests of cross-linked matrix does not react with Type II collagen antibodie

Figure 6



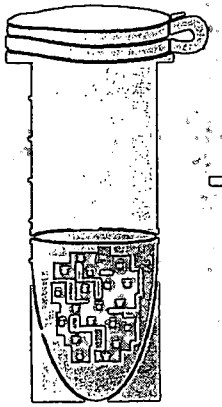
- Cross-linked matrix retains 95% hydraulic capacity

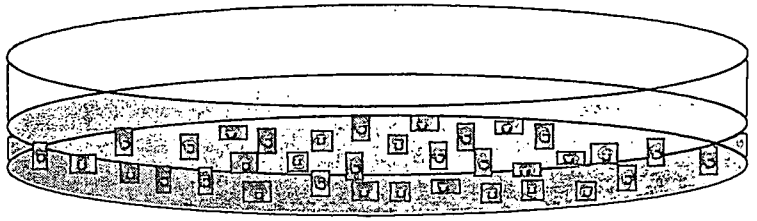
Figure 7: Growth and Proliferation of Disc cells into Cross-linked Matrix

Cell Isolation

Culture Expansion

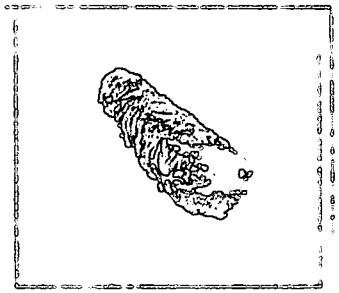
Sterile nucleus pulposus from sheep spine; enzymatic digestion for cell isolation



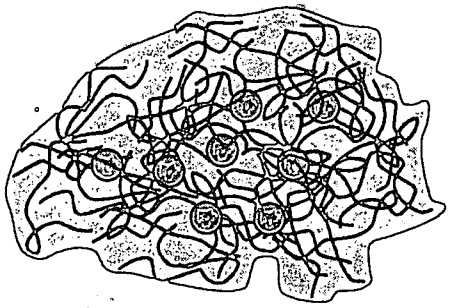


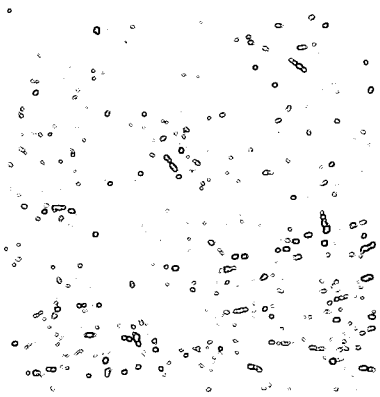
Disc Cell Culture in Cross-linked Matrix/BP

Cross-linked matrix



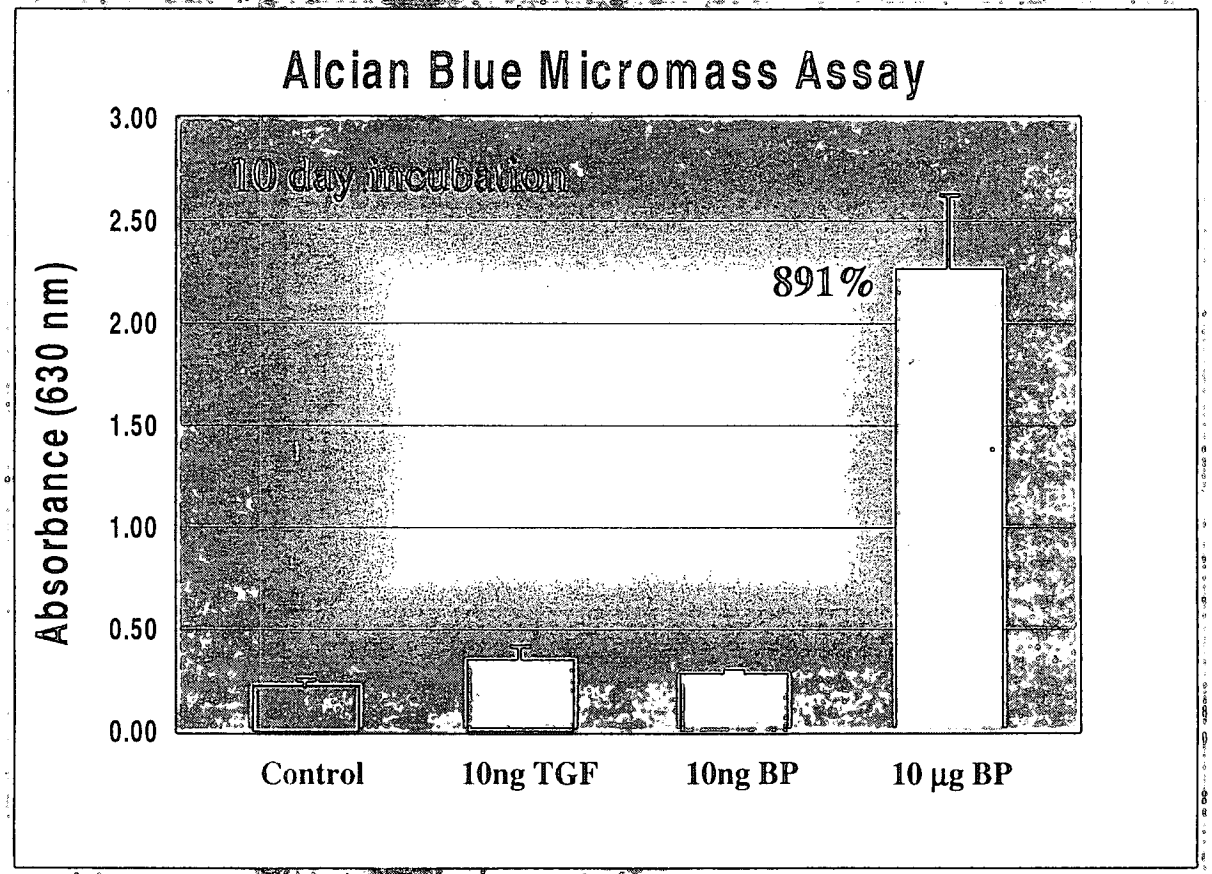
Cross-linked matrix/BP





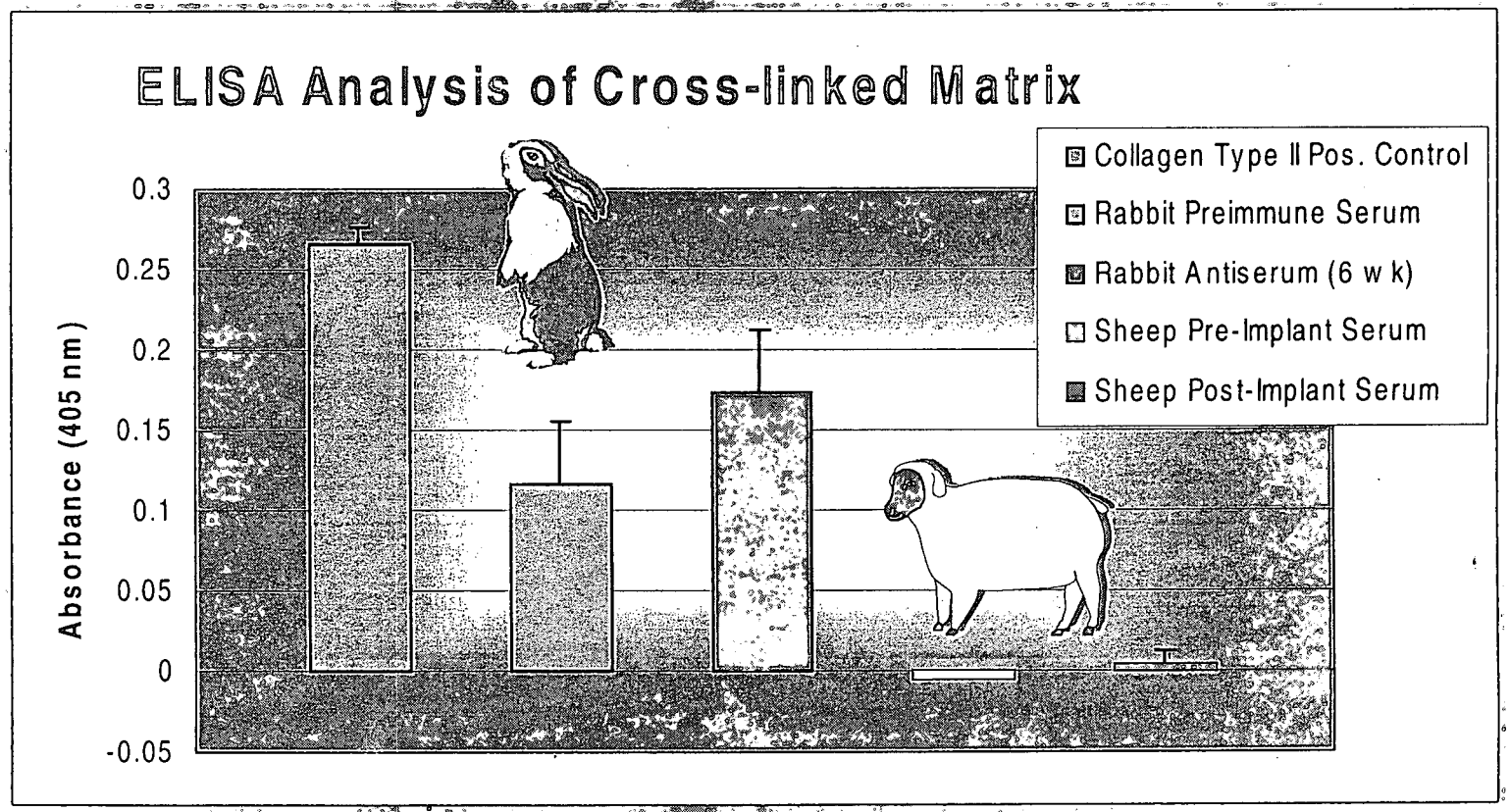
Cross-linked matrix seeded with disc cells & stained with phalloidin

Figure 8: Growth Factor Stimulation of Matrix Synthesis



- Significant stimulation of matrix production only at µg BP concentrations

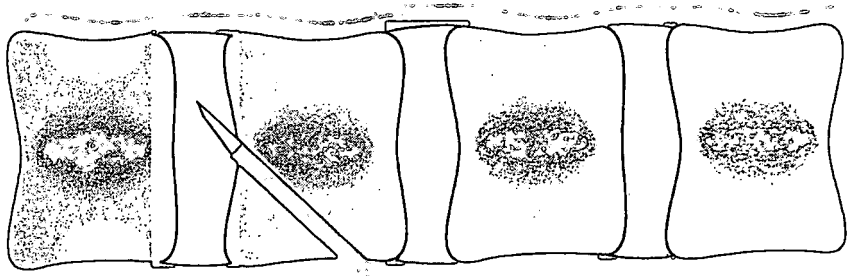
Cross-linked Matrix Immunization

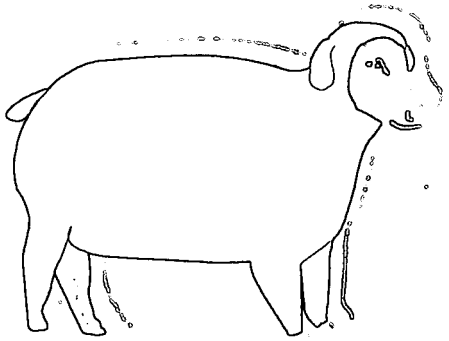


- Low antibody titers to cross-linked matrix in rabbit immunizations
- No serum antibodies to cross-linked matrix *in vivo* (1st sheep)

Study #1

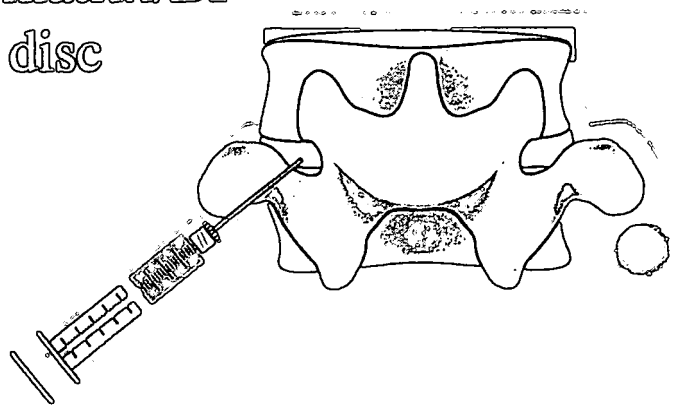
1st operation: annulus stabs to create two degenerative discs





Wait 2 months

2nd operation: Cross-linked matrix/BP gel treatment injection to one disc

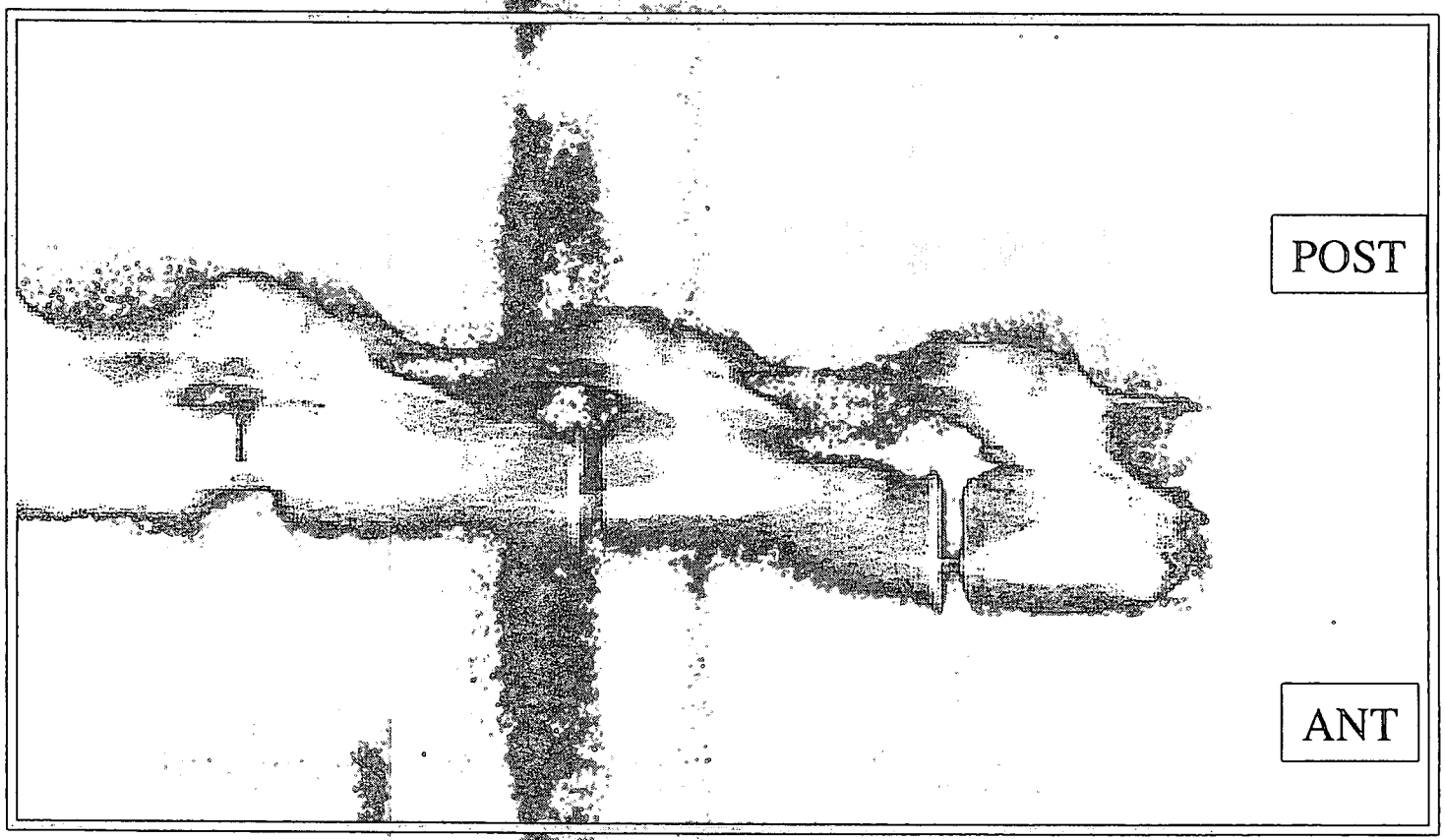


- Histomorphometry
- MRI/radiographs
- Immune response

Sacrifice: 2, 4, and 6 months

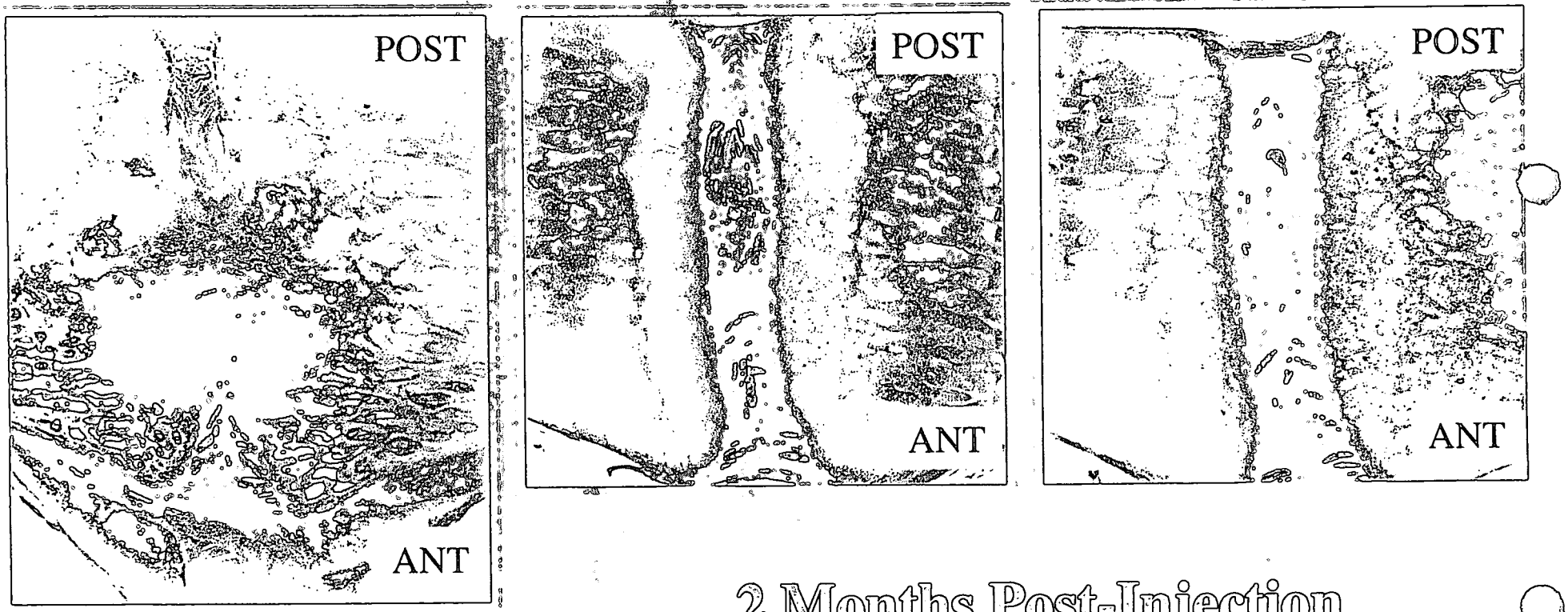
Figure 11: Radiograph-Pilot Study #1

2 Months Post Injection (Cross-linked matrix/BP)



- Treated and Control discs: normal size and appearance of disc structures
- Untreated disc: disjunct endplates, bone resorption and remodeling

Figure 12: Histology- Pilot Study #1

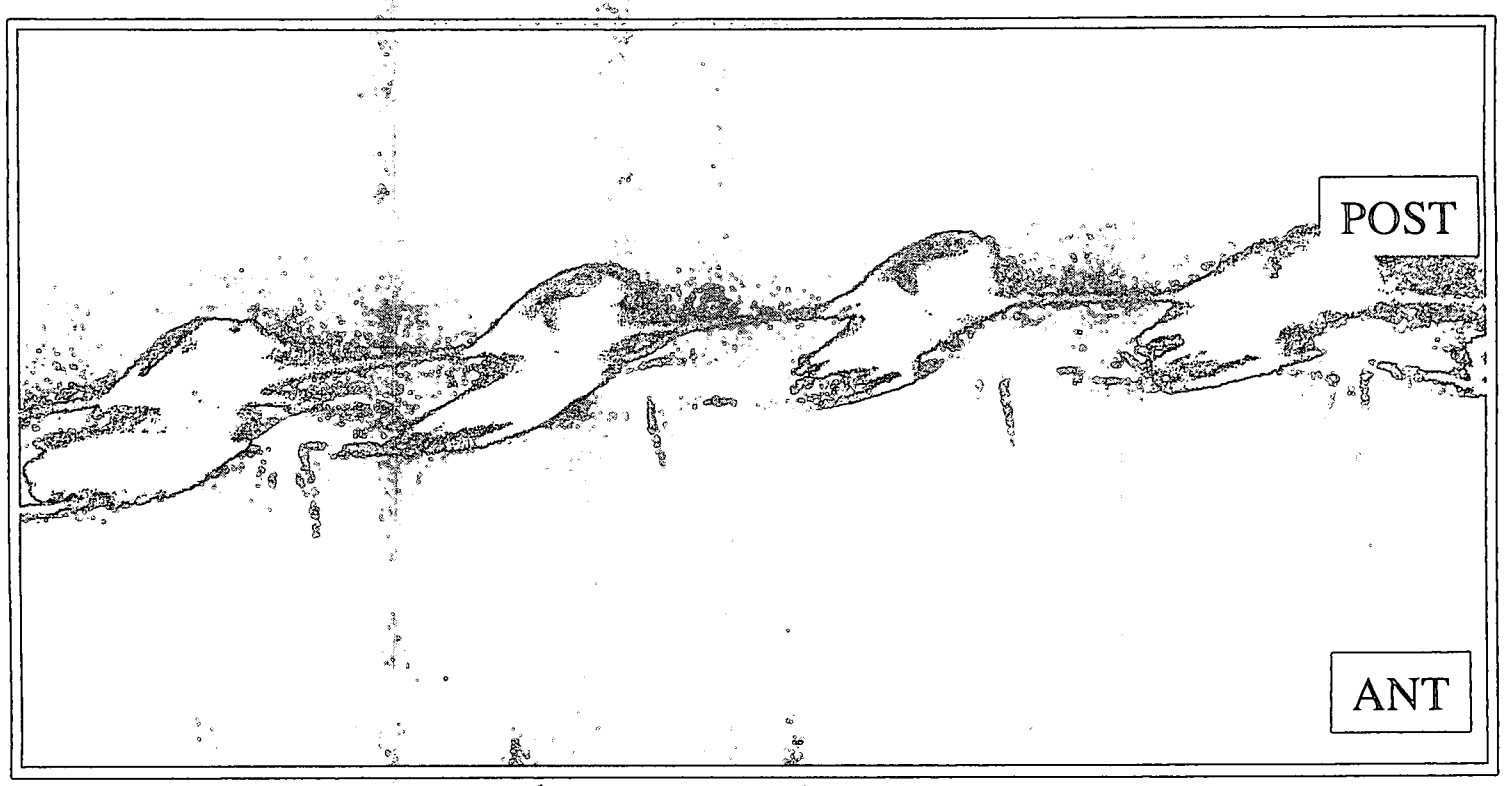


2 Months Post-Injection

- Untreated disc exhibits extensive degeneration
- Cross-linked matrix/BP-treated disc retains normal structures similar to Control disc

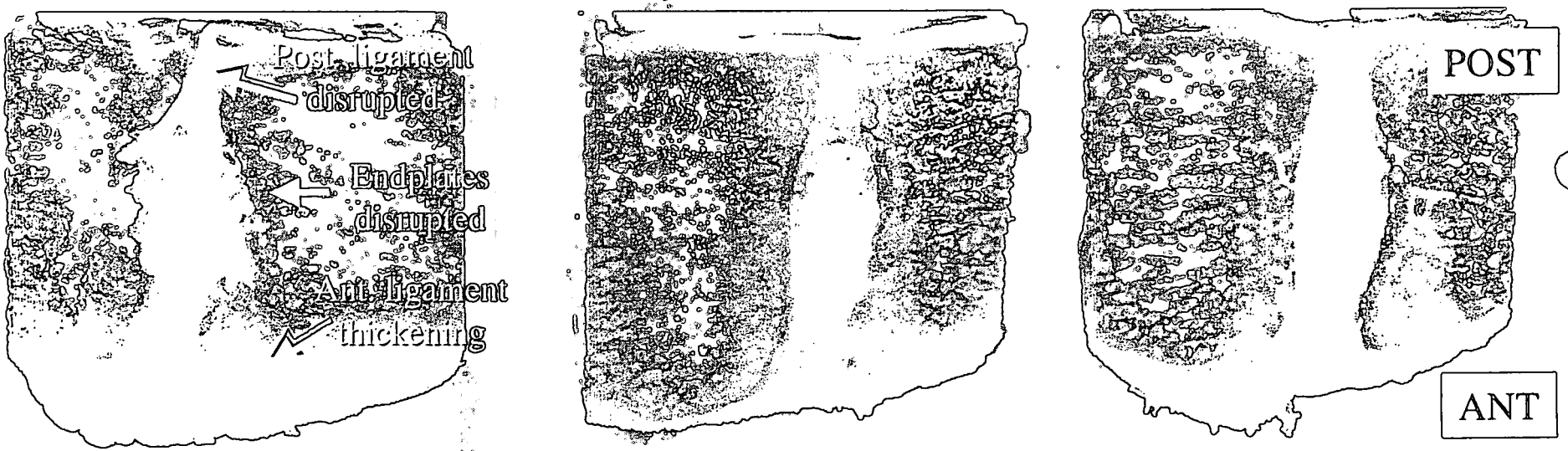
Figure 13:Radiograph- Pilot Study #1

4 Months Post Injection (Cross-linked matrix/BP)



- No apparent radiographic differences between discs in 4 month sheep.

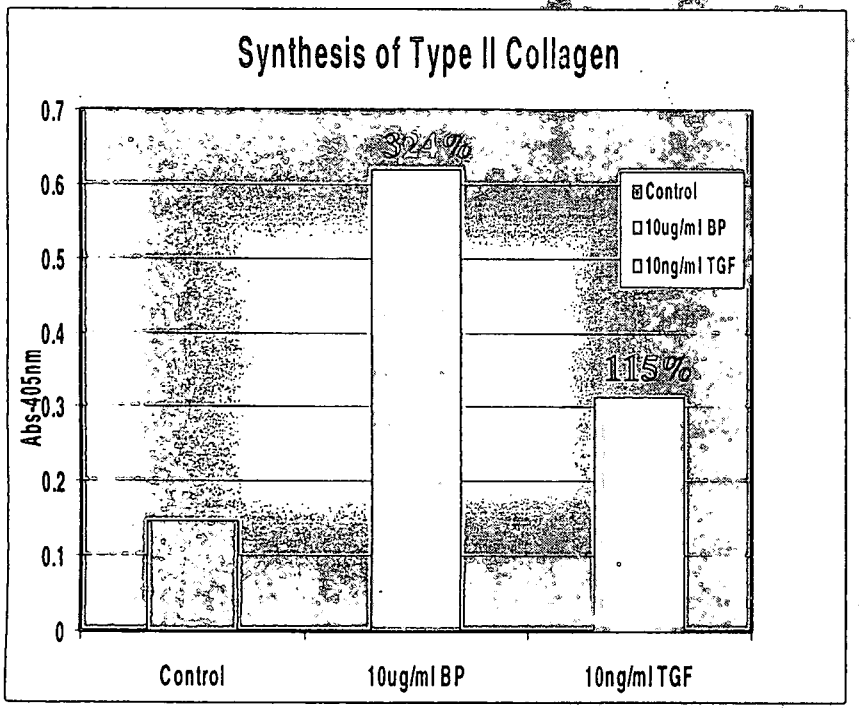
Figure 14: Histology- Pilot Study #1



4 Months Post-Injection

- Untreated disc exhibits degenerative changes
- Cross-linked matrix/BP-treated disc similar to control disc: normal gelatinous nucleus, regular annulus, intact endplates

Figure 15: Growth Factor Stimulation of Type II Collagen & Chondroitin-6-Sulfate Synthesis



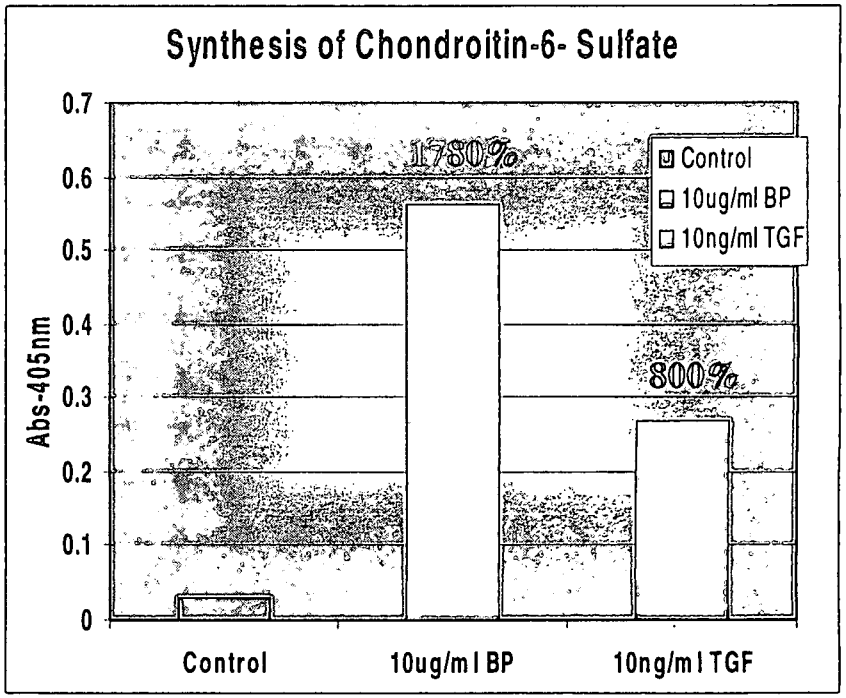


Figure 16: Growth Factor Stimulation of Proteoglycan Synthesis in Human Intervertebral Disc Nucleus Pulposus Cells

Figure 16a

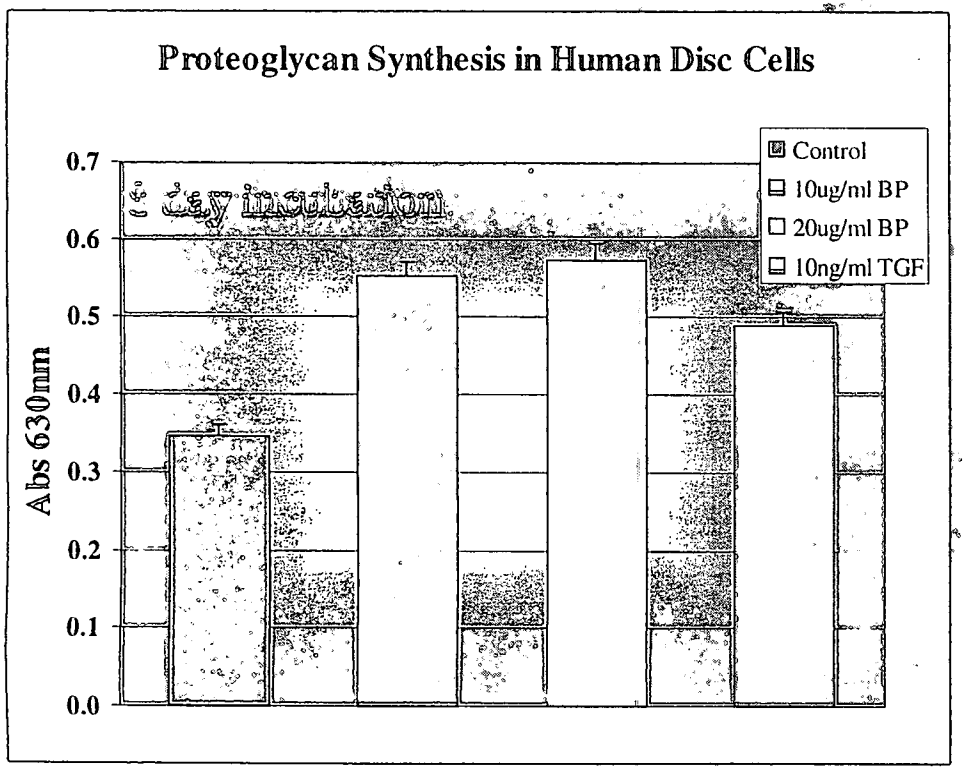


Figure 16b

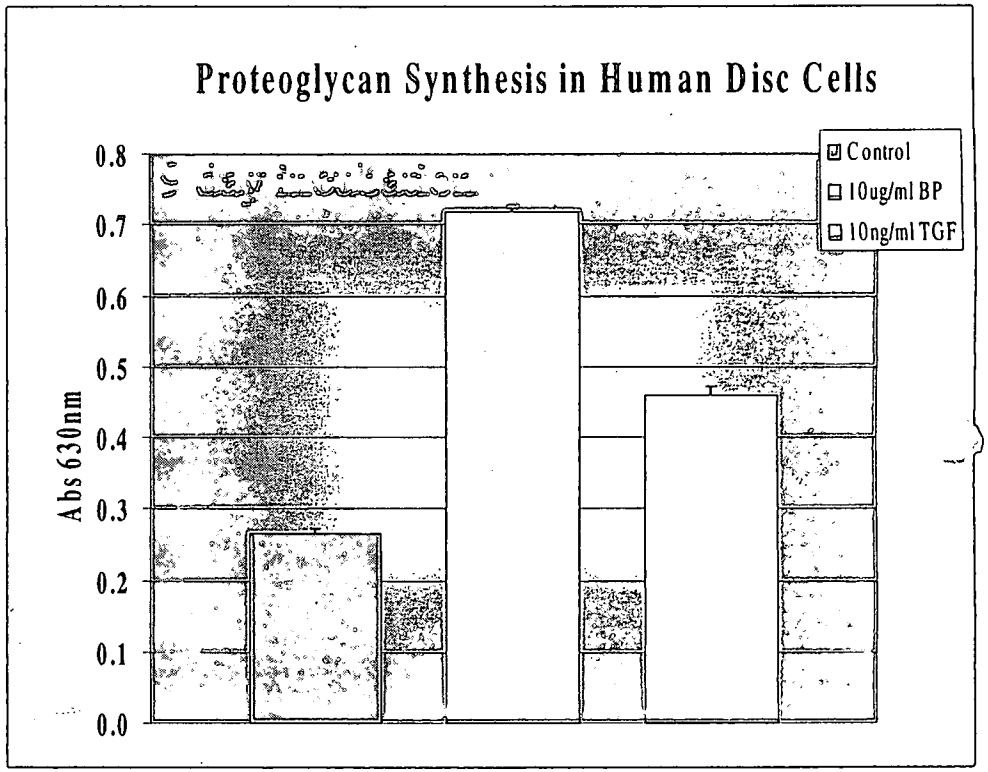


Figure 17: Growth Factor Stimulation of Proteoglycan Synthesis in Baboon Intervertebral Disc Nucleus Pulposus Cells

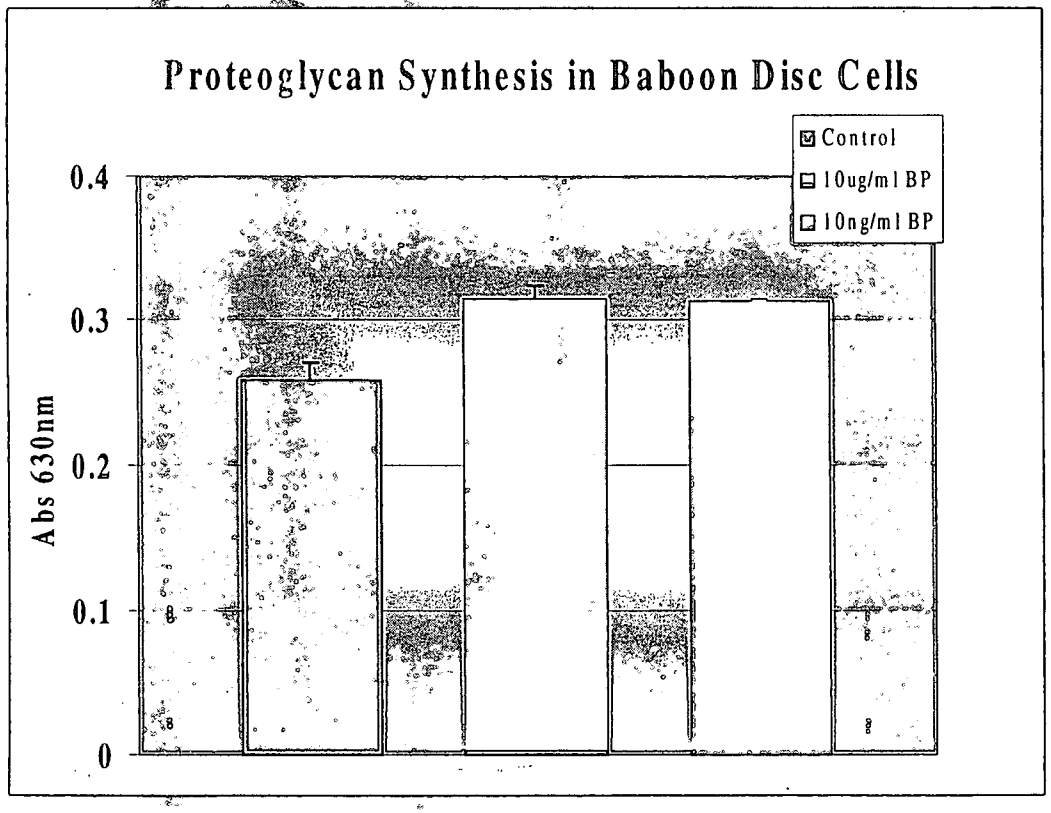
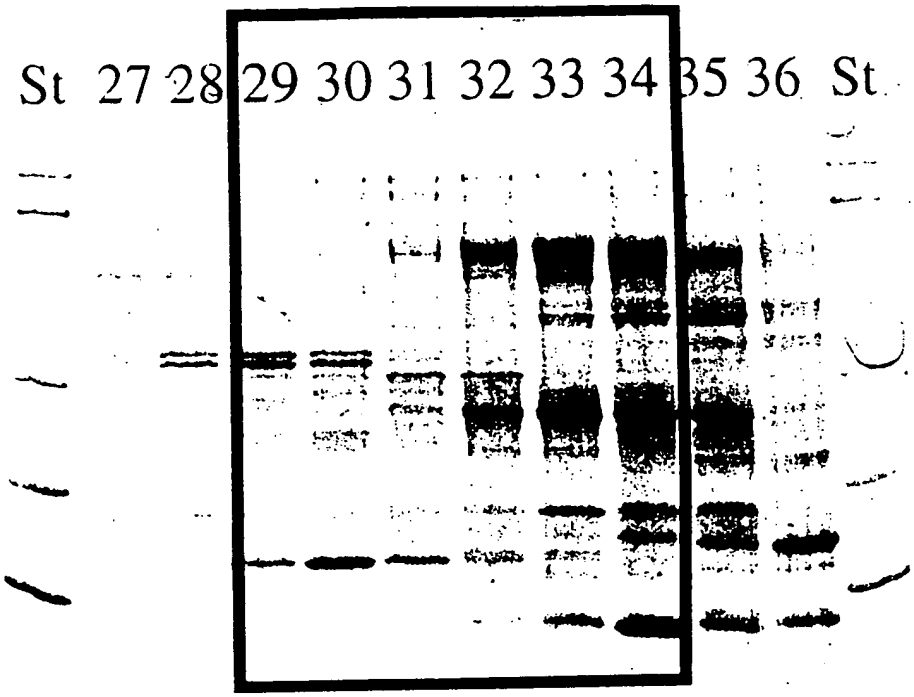


FIGURE 18



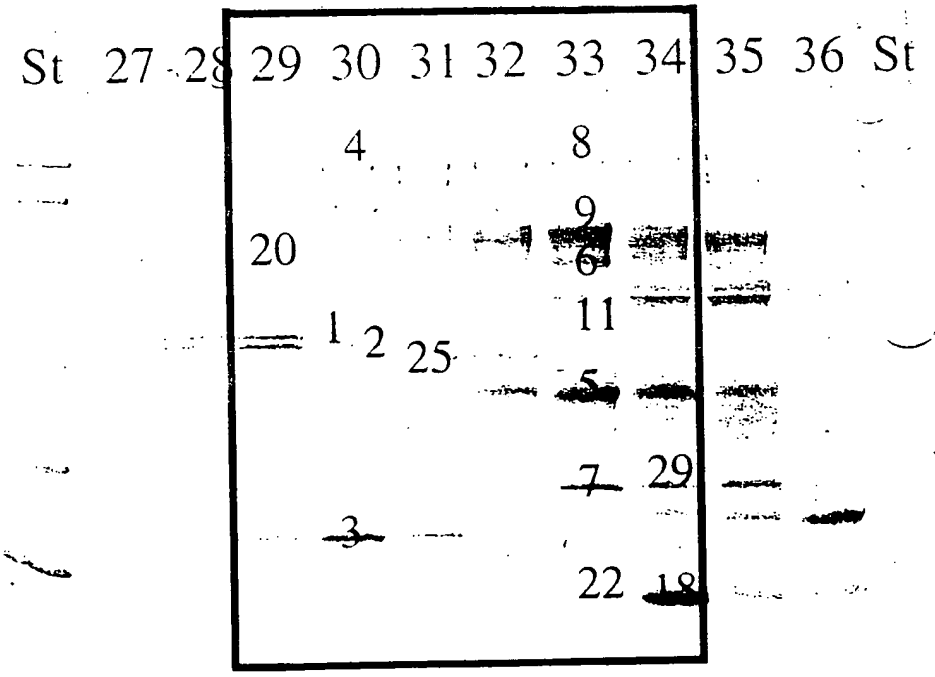
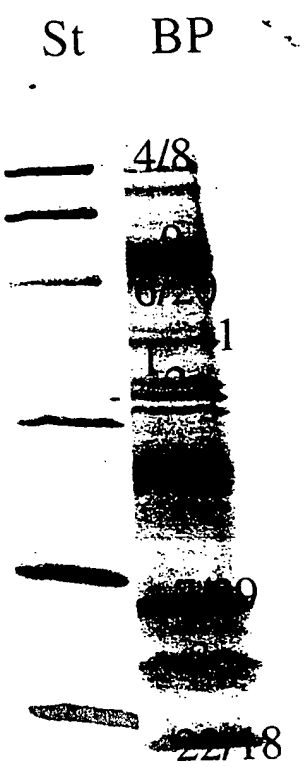


FIGURE 19



Band No.	Identity
1	histone H1.c
2	histone H1.c
3	ribosomal protein RS20
4	similar to ribosomal protein LORP
5	BMP-3
6	α2 macroglobulin RAP & BMP-3
7	similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	ribosomal protein RL6 & BMP-3
18	TGF-β2/SPP24
20	Factor H
22	TGF-β2
25	BMP-3 & H1.x
29	BMP-3 & ribosomal protein RL32

FIGURE 20

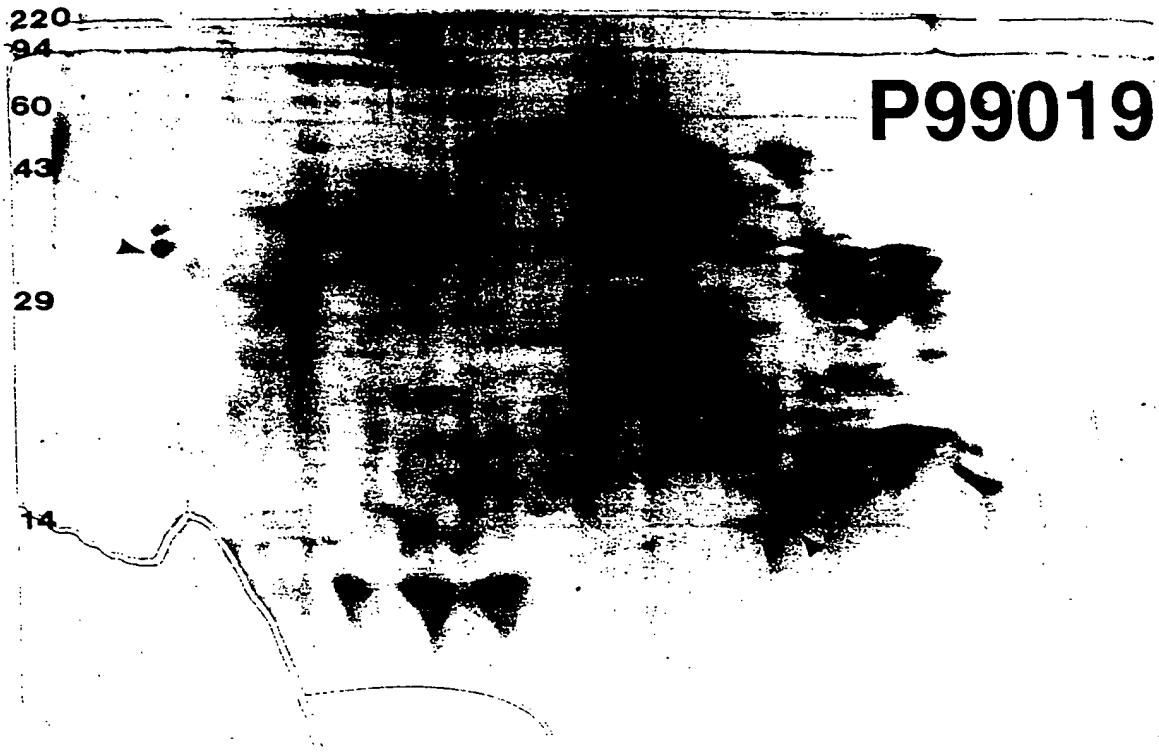
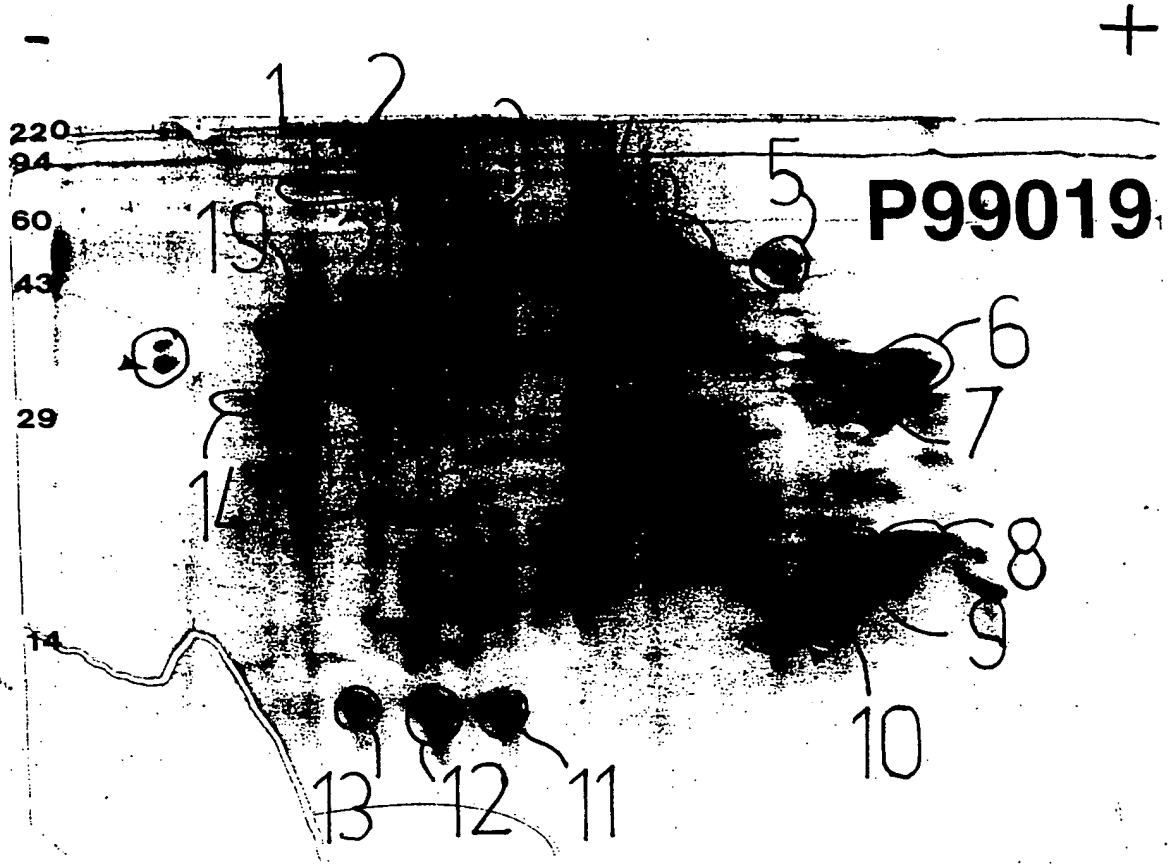


FIGURE 21



No.	Identity	No.	Identity
1	Factor XIII	11	TGF-β2/SPP24
2	LORP	12	SPP24
3	LORP	12	TGF-β2/SPP24
4		14	lysyl oxidase
5	RL3	15	lysyl oxidase
6		16	lysyl oxidase
7		17	lysyl oxidase
8		18	BMP-3
9		19	cathepsin L
10		20	
		21	RS3a

FIGURE 22

Figure 23A. (Band 1)

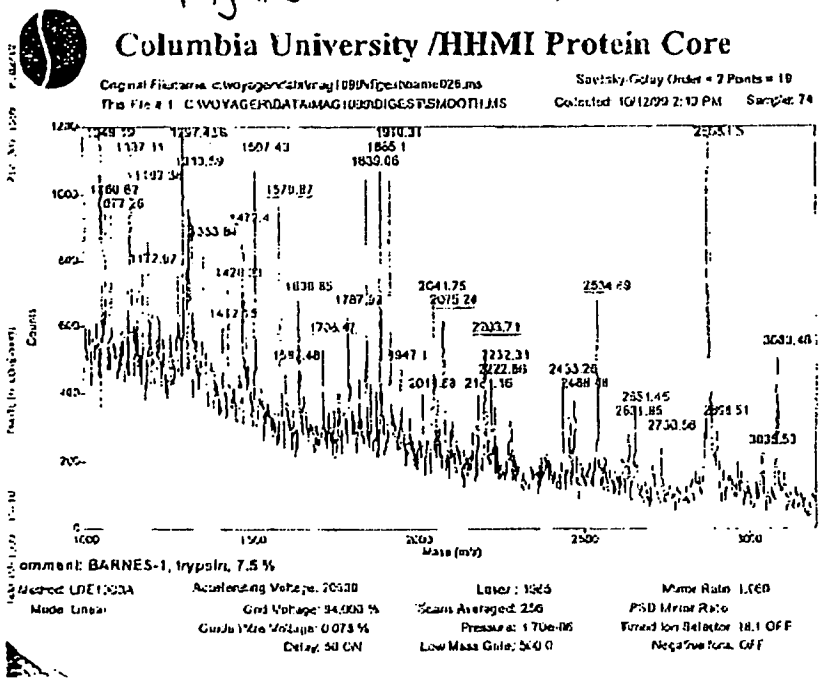
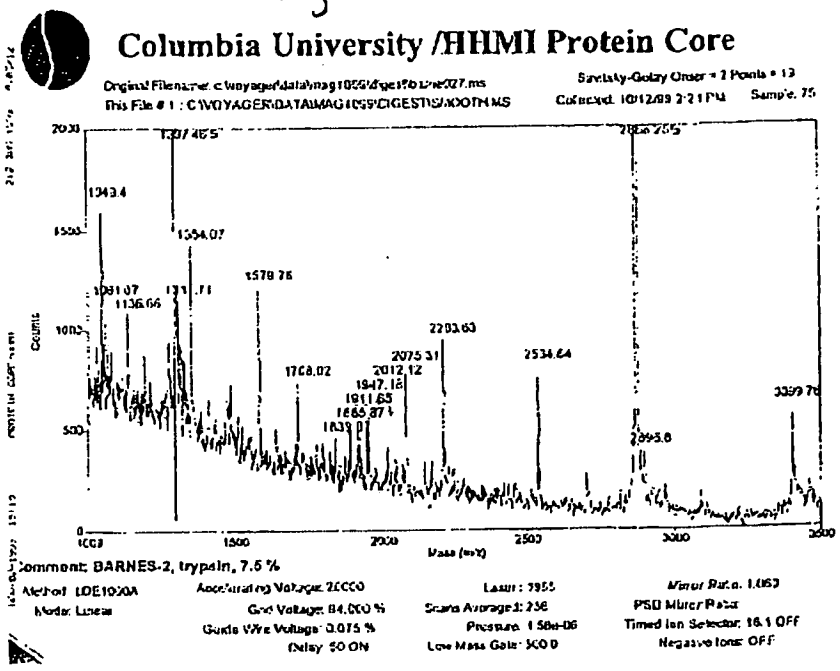


Figure 23B (Band 2)



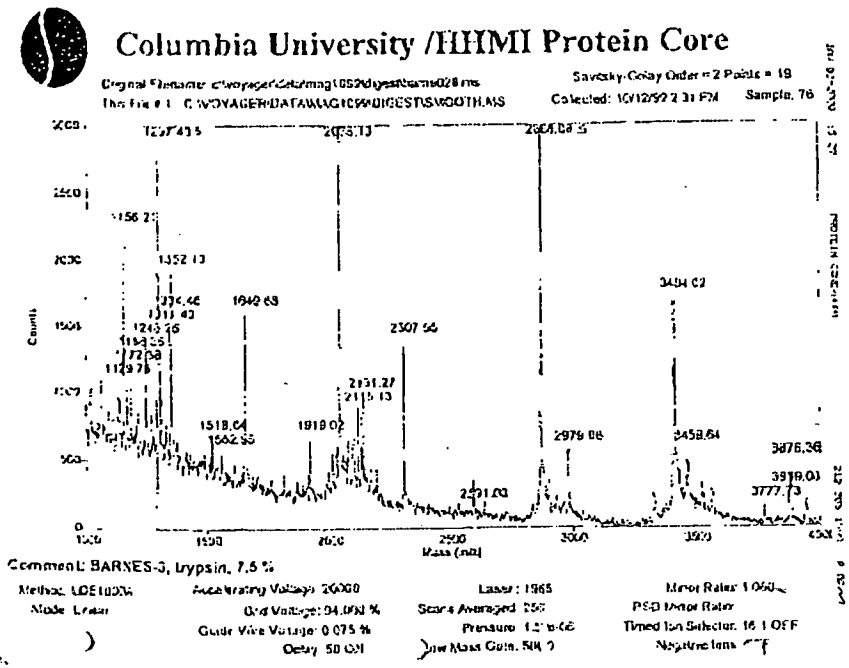


Figure 23C (Band 3)

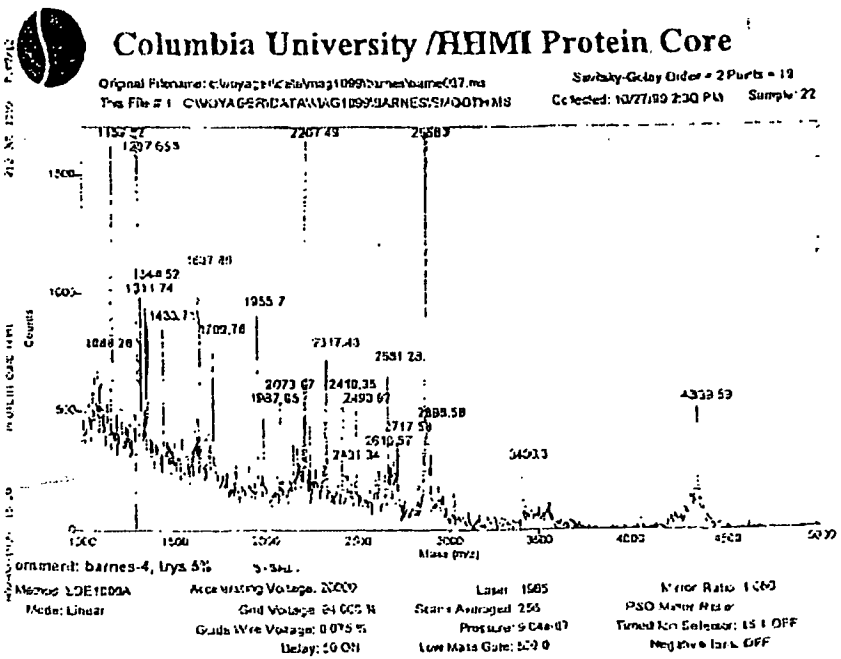


Figure 23D (Band 4)

Figure 23E (Band 5)

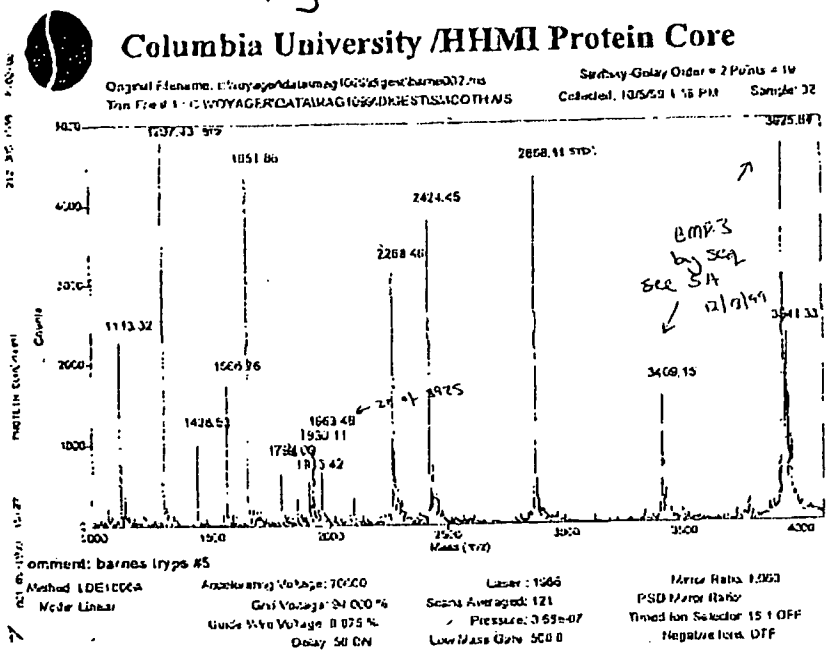
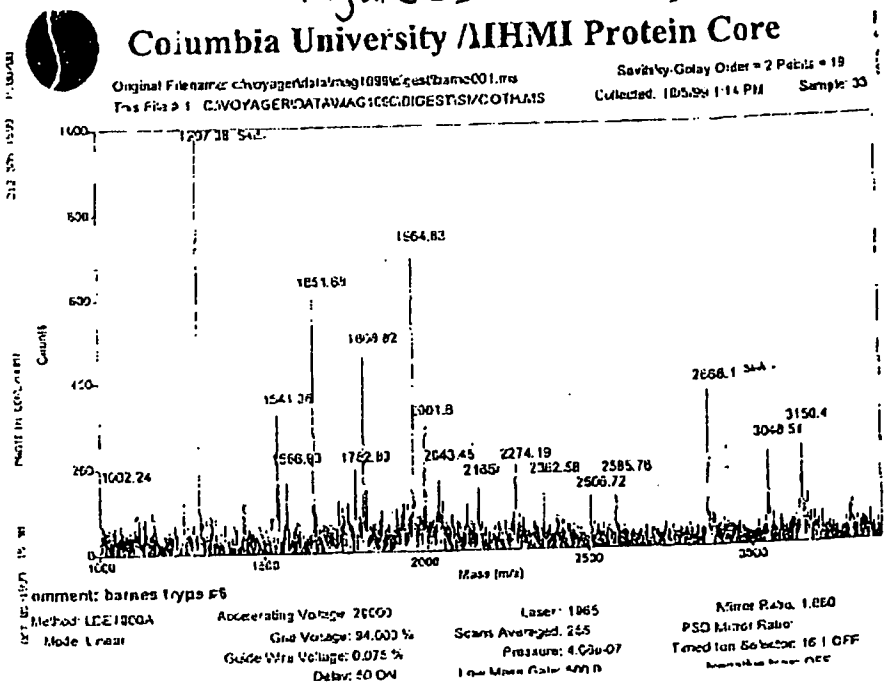


Figure 23F (Band 6)



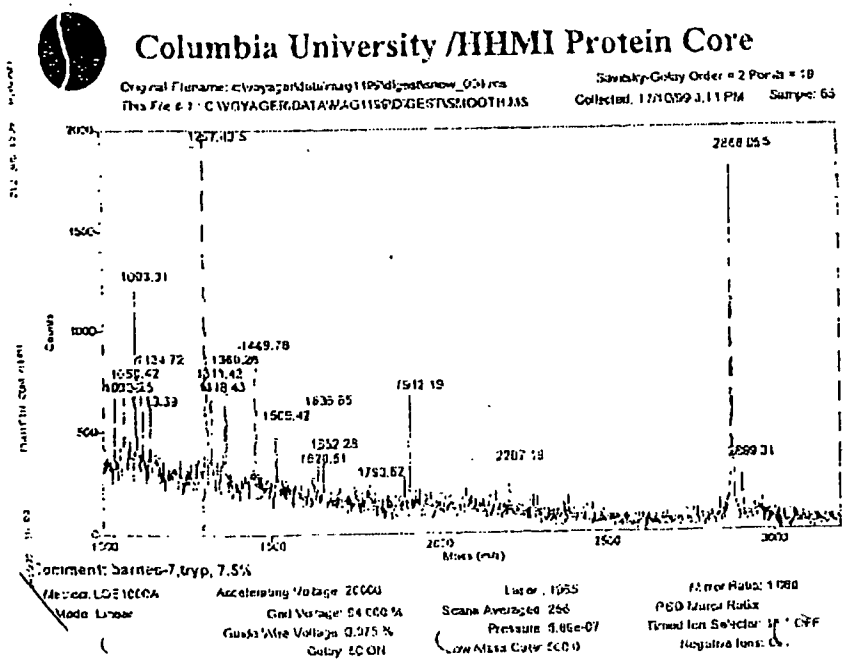


Figure 23G (Band 7)

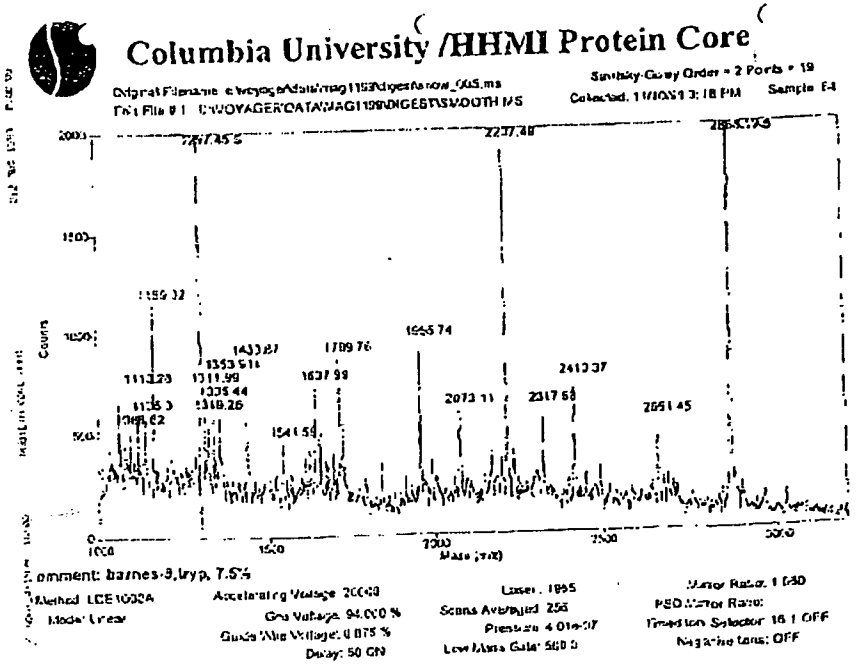


Figure 23H (Band 8)

Figure 23I (Band 9)

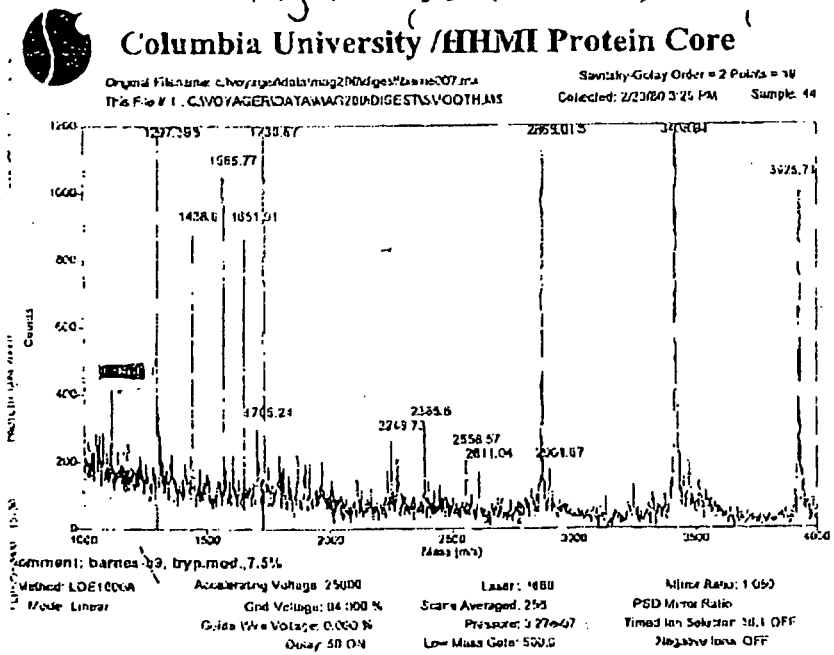
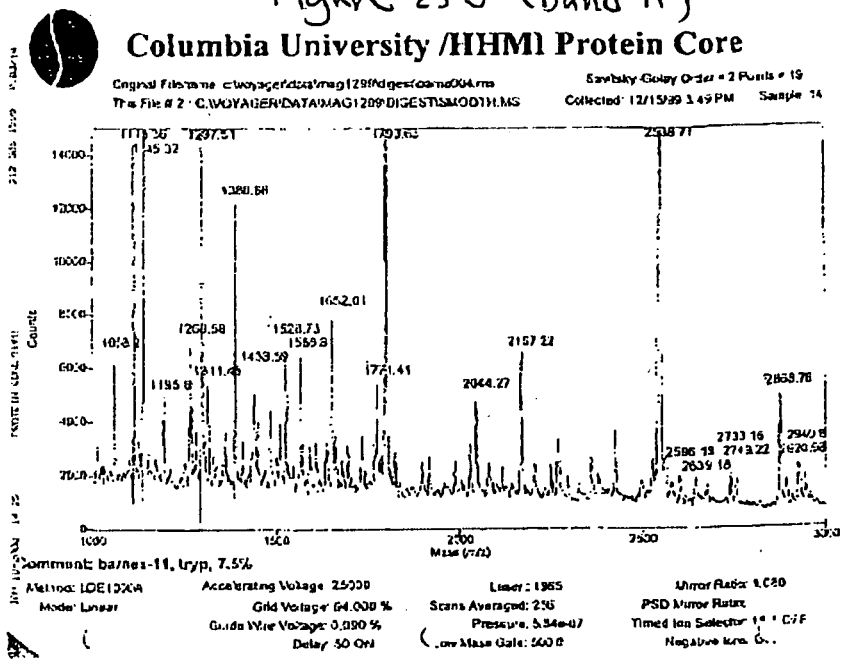


Figure 23J (Band 11)



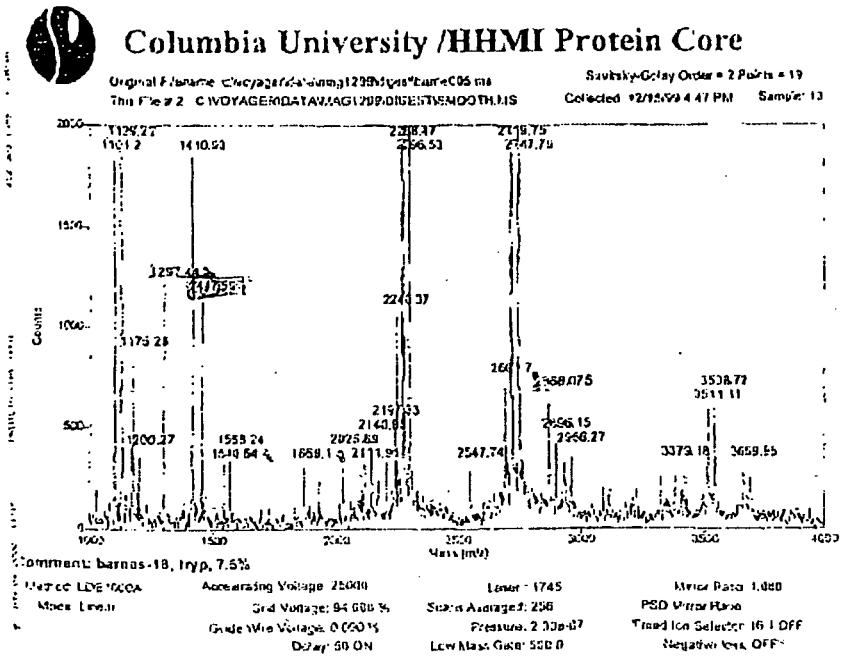


Figure 23K (Band 18)

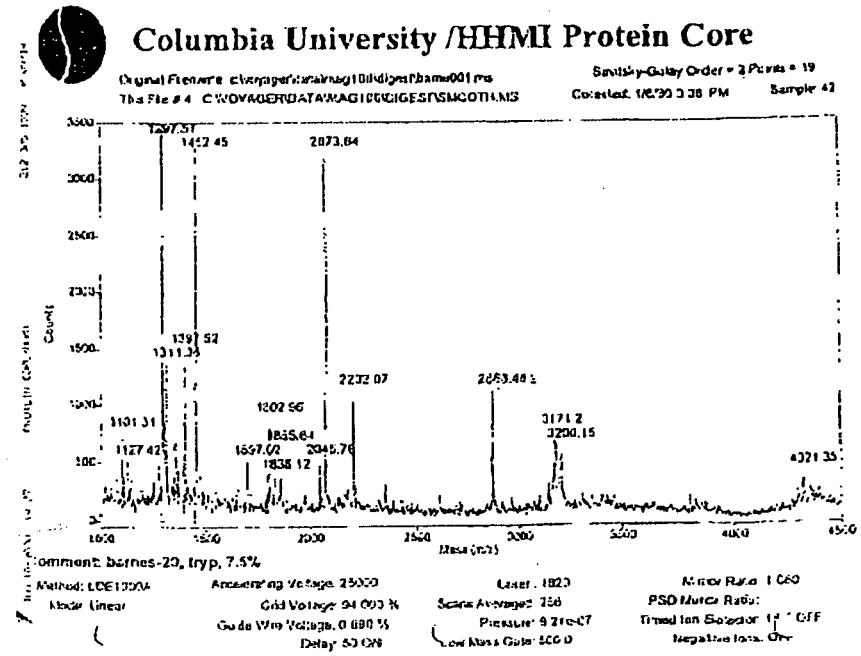


Figure 23L (Band 20)

Figure 23M (Band 22)

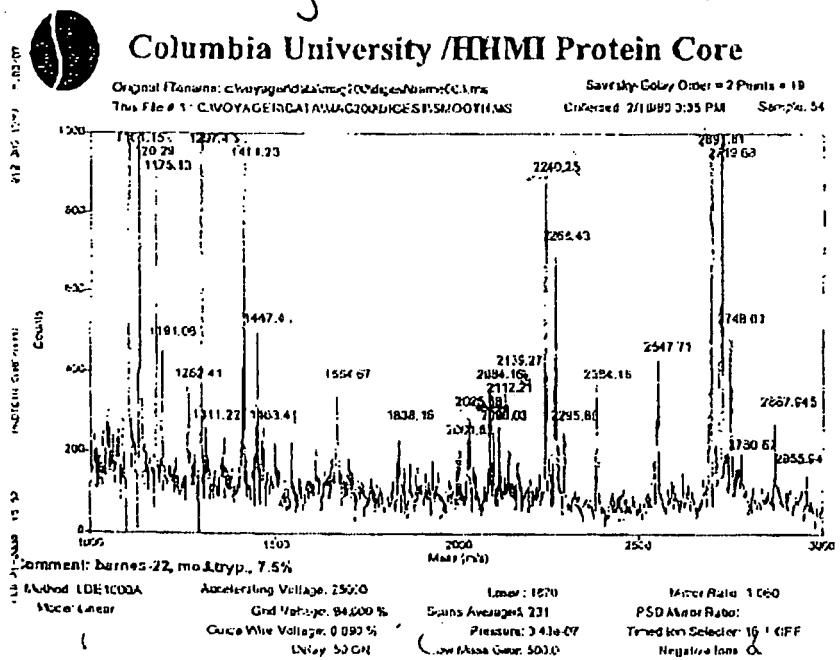
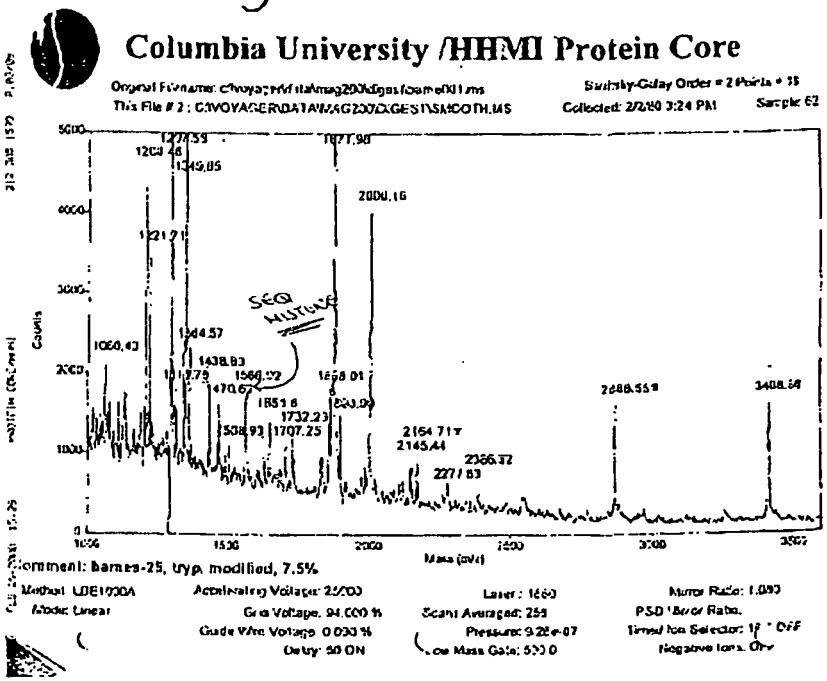


Figure 23N (Band 25)



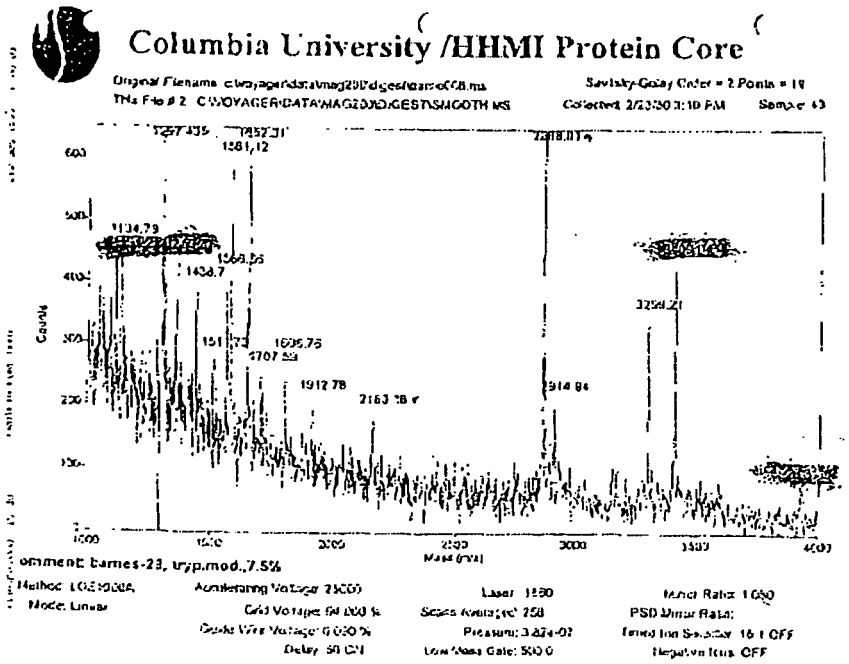


Figure 23O (Band 29)

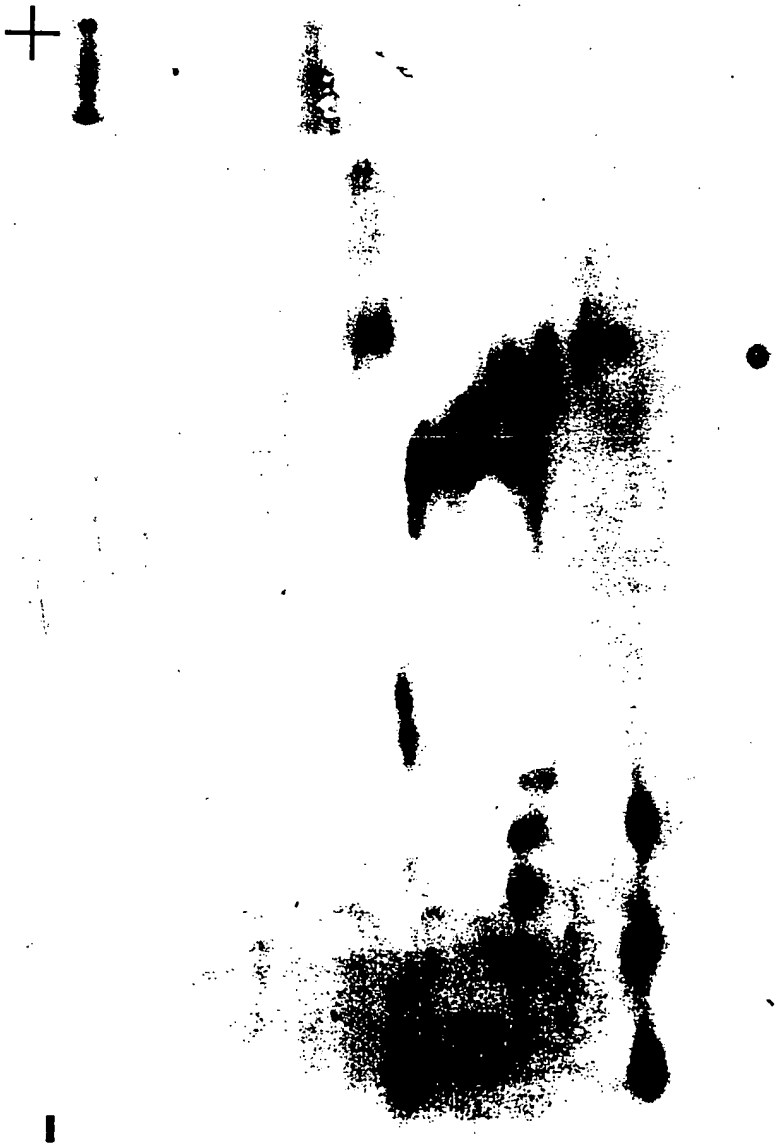


FIGURE 24

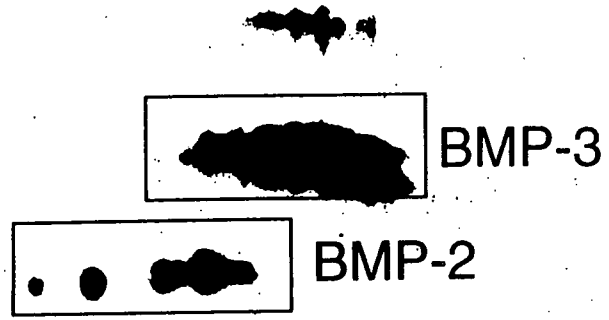


FIGURE 25A

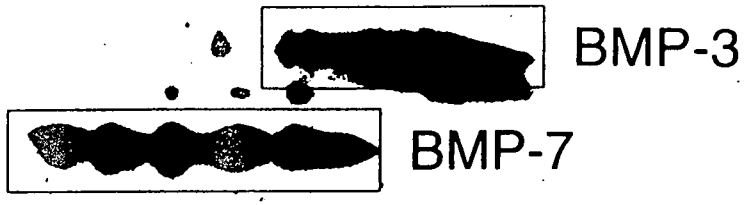


FIGURE 25B

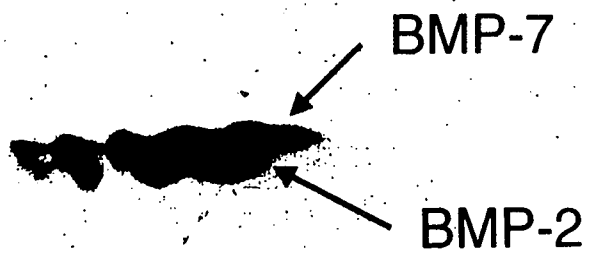


FIGURE 25C

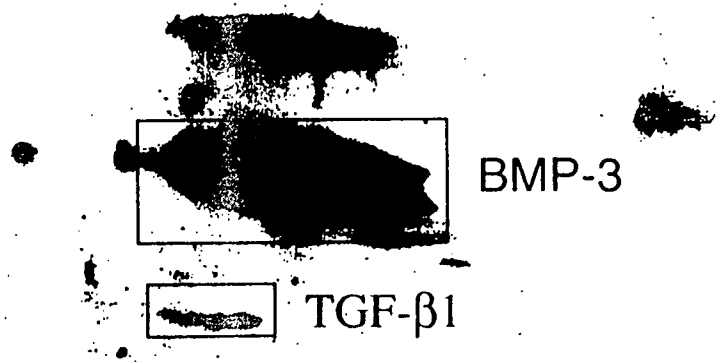
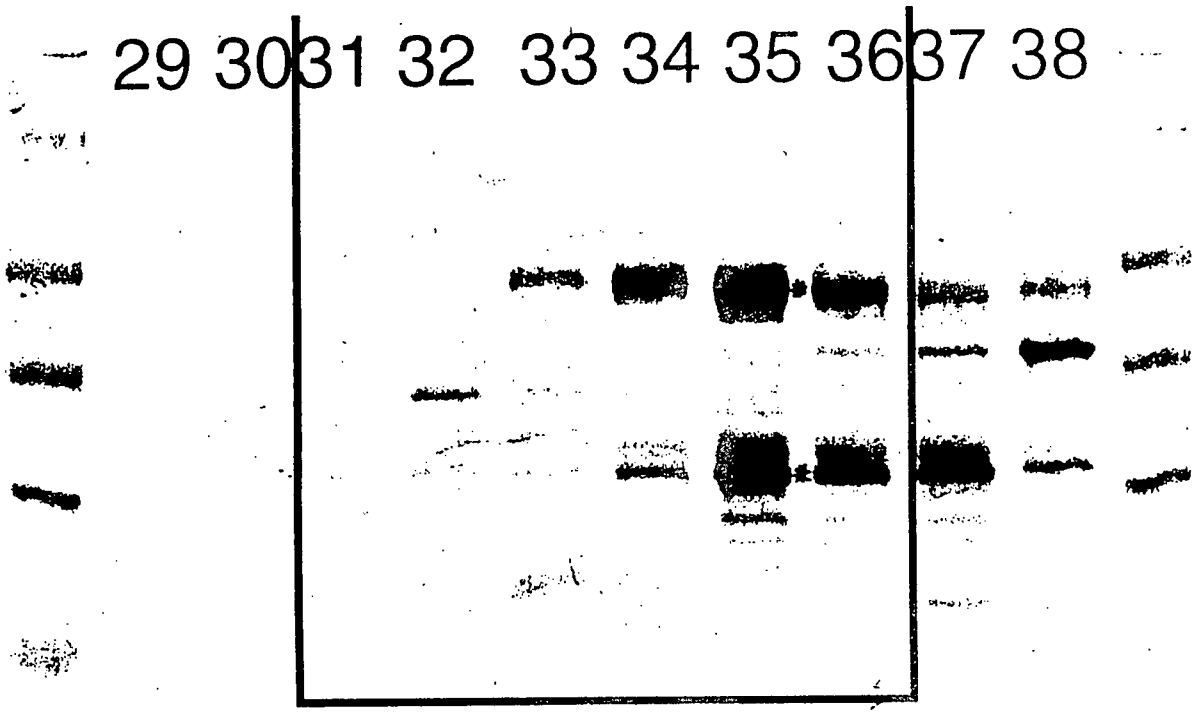


FIGURE 25D

FIGURE 26



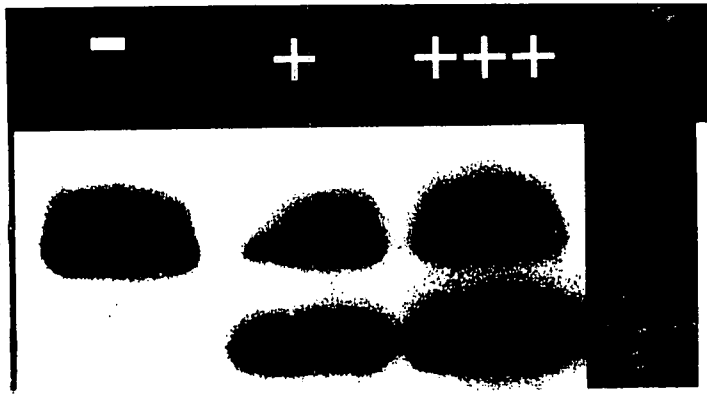


FIGURE 27

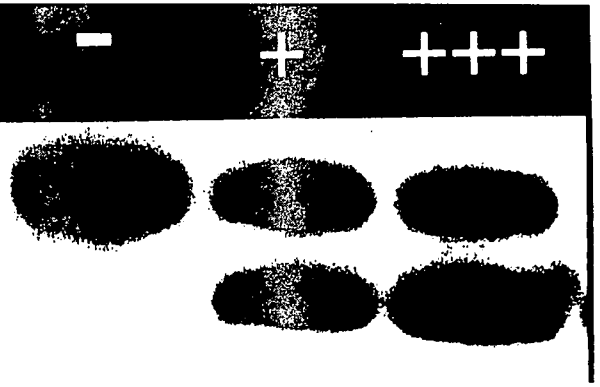
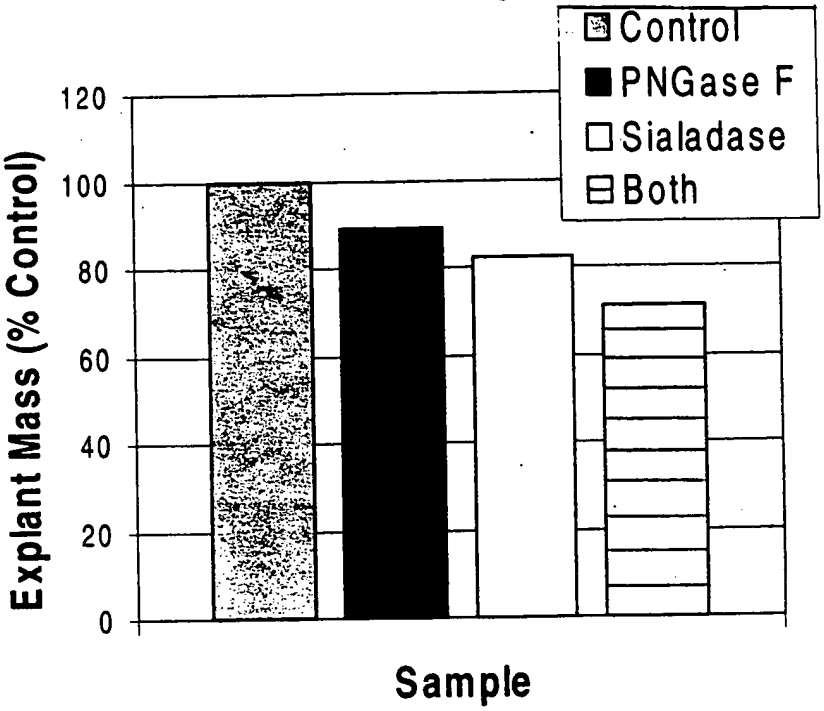


FIGURE 28

FIGURE 29A



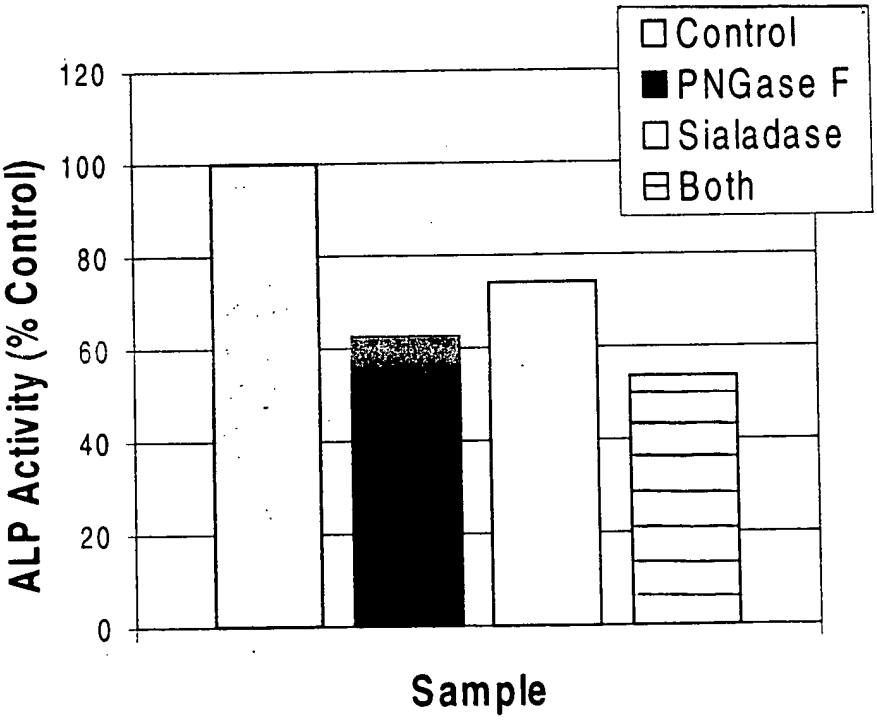


FIGURE 29B

FIGURE 30

Antibody Information

Specificity	Antigen	Host Species	PC/MC	Source	Catalog No.
TGF-β1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221
TGF-β2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90
TGF-β3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-6896
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novocastra Laboratories	NCL-BMP6
BMP-7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA
FGF-1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884
osteonectin (bovine)	Protein	Mouse	Monoclonal	DSHB	AON-1
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R1H
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740

Figure 31A. Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Acc. No.	AAs
1								
2	fx 49 (1579)	XLAAAGYDVEK	ALAAAGYDVEK	11/11	histone H1.c	human	87668 (NCBI)	65-75
3	fx 67 (1346)	SLEKVCADLIR	SLEKVCADLIR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4	fx 65 ()	(V)VCGMLGFP**SEA**PV	VVCGMLGFP**GEKR**V	11/14	LORP	mouse	AAC95338 (NCBI)	213-226
5	N terminal seq	STGVLLPLQNNELPG	STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290-304
	fx 72 (3925)	STGVLLPLQNNELPGA EYQY	STGVLLPLQNNELPG AEYQY	20/20	BMP-3	human	4557371 (NCBI)	290-309
	fx 74 (3409)	STGVLLPLQ	STGVLLPLQ	9/9	BMP-3	human	4557371 (NCBI)	290-298
6	fx 55 (1566)	(S)QTLQF**X**E	SQTLQF**D**E	7/8	BMP-3	human	4557371 (NCBI)	346-353
	fx 47	VYAF	no match		???			
	N terminal seq	HAGKYSREKNT(P)A(P)	HGGKYSREKNQPKP	11/14	α2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46
	fx 57 (1438)	SQTLQFDEQ	SQTLQFDEQ	9/9	BMP-3	human	4557371 (NCBI)	346-354
	fx 57 (1652)	SLKPSNHA	SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410-417
7	fx 51 (1093)	AALRPLVKP	AALRPLVKP	9/9	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	1-9
	fx 37 (no MS)	A(H)I(Q)VERYV	AIVER	5/5	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	109-113
	fx 37 (no MS)	A(H)I(Q)VERYV	HQ**S**DRYV	5/7	60s Ribosomal Protein L32	mouse	P17932 (Swiss-Prot)	22-28
8	fx 78 ()	XALF(G)AQLGXALGPI	no match		???			
9	fx 56 (1567)	SQTLQFDEQT	SQTLQFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346-355

Figure 31B. Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Acc. No.	AAs
11	fx 55 (1311)	SQTLXF	SQTLQF	5/6	BMP-3	human	4557371 (NCBI)	346-351
	fx 47 (1772)	VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	87-99
	fx 76 (1795)	xVFAL	VFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
	fx 61 (1145)	AVPQLQGYLR	AIPQLQGYLR	9/10	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	262-271
18								
22	fx 58 (1101)	ALDAAYCFR	ALDAAYCFR	9/9	TGF-β2	human	P08112 (Swiss-Prot)	303-311
	fx 69 (no match)	GYNANFCAGACPYL	GYNANFCAGACPYL	14/14	TGF-β2	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71)	VNSQSLSPY	VNSQSLSPY	9/9	SPP24	bovine	Q27967 (Swiss-Prot)	42-50
25	fx 39 (1470)	KAAKPSV(P)	KAAKPSVP	8/8	Histone H1.x	human	JC4928 (PIR)	199-206
29								

fx = fraction number (molecular weight of fragment, as measured by SDS-PAGE)

Figure32A. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
1	4 peaks match with histone H1.c	human	87668 (NCBI)	1172.97	1172.37	0.60	110-121	22	15 MS peaks match with Band 2
				1579.87	1579.71	0.16	65-79		
				1708.47	1707.89	0.58	64-79		
				2011.58	2012.32	-0.74	35-54		
2	3 peaks match with histone H1.c	human	87668 (NCBI)	1579.76	1579.71	0.05	65-79*	16	identification of starred peptide confirmed by sequence analysis
				1708.02	1707.89	0.13	64-79		
				2012.12	2012.32	-0.20	35-54		15 MS peaks match with Band 1
3	7 peaks match with ribosome S20	rat	R3RT20 (PIR)	1129.76	1129.40	0.36	50-59	62	
				1156.21	1156.30	-0.09	76-83		
				1334.46	1334.62	-0.16	56-66		
				1352.13	1351.58	0.55	88-99		
				1518.04	1517.77	0.27	9-21		
				1919.02	1919.19	-0.17	5-21		
				3404.02	3404.87	-0.85	88-119		
4	3 peaks match with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	1987.95	1988.27	-0.32	150-167	8	12 MS peaks match with Band 8
				2410.35	2410.63	-0.28	648-669		
				2610.57	2610.10	0.47	455-478		

Figure32B. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
5	9 peaks match with BMP-3	human	4557371 (NCBI)	1113.32	1113.31	0.01	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.53	1438.58	-0.05	346-357		
				1566.76	1566.76	0.00	345-357		
				1651.86	1651.91	-0.05	410-424		
				1794.09	1794.02	0.07	346-360		
				2268.46	2268.63	-0.17	374-392		
				2424.45	2424.81	-0.36	373-392		identification of starred peptide confirmed by sequence analysis
				3409.15	3407.77	1.38	290-318*		
6	3 peaks match with α2-Macroglobulin RAP	human	P30533 (Swiss-Prot)	1002.24	1002.15	0.09	283-290	17	
				2362.58	2362.43	0.15	129-150		
				3048.51	3048.52	-0.01	257-282		
	2 peaks match with BMP-3	human	4557371 (NCBI)	1566.93	1566.75	0.18	346-357	15	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1651.88	1651.91	-0.03	410-424		

Figure32C. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
7	4 peaks match with ribosome L32	mouse	P17932 (Swiss-Prot)	1033.25	1033.17	0.08	67-75	33	
				1093.31	1093.40	-0.09	1-10*		
				1134.72	1134.28	0.44	65-74		
				1449.78	1449.66	0.12	19-29		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1060.42	1060.20	0.22	102-111	21	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1113.39	1113.31	0.08	361-368		
				1360.26	1360.58	-0.32	190-200		
				1652.28	1651.91	0.37	410-424		
				1793.62	1794.02	-0.40	346-360		
8	1 peak matches with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	2410.37	2410.63	-0.26	648-669	3	12 MS peaks match with Band 4
9	6 peaks match with BMP-3	human	4557371 (NCBI)	1113.14	1113.31	-0.17	361-368	36	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.60	1438.58	0.02	346-357		
				1566.77	1566.76	0.01	345-357		
				1651.91	1651.61	0.30	410-424		
				2901.67	2901.19	0.48	41-66		
				3408.94	3407.77	1.17	290-318		

Figure32 D. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
11	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.23	1113.31	-0.08	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1651.73	1651.91	-0.18	410-424		
				1793.58	1794.02	-0.44	346-360		
				2424.24	2424.81	-0.57	373-392		
				3408.34	3407.77	0.57	290-318		
	5 peaks match with ribosome L6	human	Q02878 (Swiss-Prot)	1140.38	1140.23	0.15	114-122	16	
				1526.88	1526.86	0.02	141-155		
		mouse	P47911 (Swiss-Prot)	1059.15	1059.12	0.03	10-20		
				1145.36	1145.35	0.01	262-271		
				1386.74	1386.68	0.06	260-271		
18	4 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1101.20	1101.26	-0.06	303-311	52	
				1175.26	1175.42	-0.16	400-409		
				2240.37	2240.60	-0.23	312-328		
				2691.70	2691.91	-0.21	340-362		
	5 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1410.93	1411.60	-0.67	42-53	30	
				1447.59	1447.65	-0.06	113-124		
				1540.64	1540.60	0.04	86-98		
				1869.10	1869.05	0.05	62-77		
				2268.47	2268.57	-0.10	33-53		

Figure32 E. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
22	5 peaks match with TGF-β2	human	P08112 (Swiss-Prot)	1101.15	1101.26	-0.11	303-311	63	
				1175.13	1175.42	-0.29	400-409		
				2084.16	2084.42	-0.26	312-347		
				2240.25	2240.60	-0.35	312-328		
				2691.61	2691.91	-0.30	340-362		
	2 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1411.23	1411.60	-0.37	42-53	11	
				1447.40	1447.65	-0.25	113-124		
25	5 peaks match with histone H1.x	human	JC4928 (PIR)	1208.46	1208.40	0.06	48-57	14	
				1221.71	1222.35	-0.64	107-118		
				1349.85	1350.52	-0.67	107-119		
				1364.57	1364.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
	5 peaks match with BMP-3	human	4557371 (NCBI)	1060.43	1060.20	0.23	102-111	31	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.83	1438.58	0.25	346-357		
				1566.92	1566.76	0.16	345-357		
				1651.80	1651.91	-0.11	410-424		
				3408.86	3407.77	1.09	290-318		

Figure32F. Identification of Proteins by Mass Spectrometry of Tryptic Fragments

Band	Mass Spec Profile	Species	Acc. No.	Mass Spec Data	Mass Spec Database	Mass Diff-erence	AAs	% Cover-age	Comments
29	4 peaks match with BMP-3	human	4557371 (NCBI)	1113.22	1113.31	-0.09	361-368	27	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.70	1438.58	0.12	346-357		
				1566.86	1566.75	0.11	345-357		
				3409.04	3407.77	1.27	290-318		

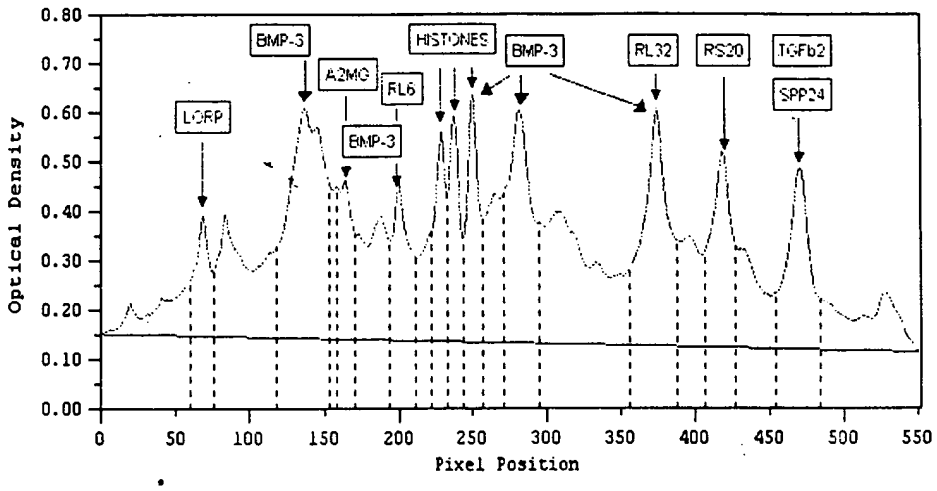


FIGURE 33A

FIGURE 33B

Figure 34. Quantitation of Identified BP proteins

Identified Protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMPO3 & A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS20	5
SPP24 & TGF-β2	6
Total	58%